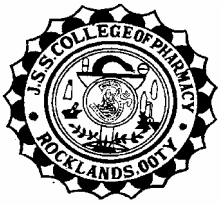
# Development and validation of *In Vitro – In Vivo* Correlations for the developed modified release formulations of selected drug candidates

Thesis submitted to
The Tamilnadu Dr.M.G.R. Medical University, Chennai, India
in partial fulfillment of the requirements
for the degree of
**Doctor of Philosophy**

**Submitted**
**by**
**S.RAJAN, M.Pharm.,**

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# DECLARATION

I hereby declare that the thesis entitled **"Development and validation of *In Vitro* – *In Vivo* Correlations for the developed modified release formulations of selected drug candidates"** submitted by me for the award of degree of **Doctor of Philosophy** of the Tamilnadu Dr.M.G.R. Medical University, Chennai is a record of research work done by me at J.S.S. College of Pharmacy, Ootacamund - 643 001, Tamilnadu, India during the years 2006-2009 under the supervision of Dr.S.N.Meyyanathan and that the thesis had not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title previously.

**S.RAJAN**

# CERTIFICATE

This is to certify that the thesis entitled **"Development and validation of *In Vitro* – *In Vivo* Correlations for the developed modified release formulations of selected drug candidates"** is a record of research work done by Mr.S.Rajan at J.S.S. College of Pharmacy, Ootacamund - 643 001, Tamilnadu, India during the years 2006-2009 under my supervision and that the thesis had not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title previously. I also certify that the thesis represents independent work done by the candidate.

**Dr.S.N.Meyyanathan**

**Supervisor**

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# CONTENTS

# 1. INTRODUCTION

This thesis deals with the investigations carried out by the writer in the laboratory for the past three years on the development and validation of *in vitro-in vivo* correlations (IVIVC) for some developed modified release formulations of selected drug candidates. Before discussing the experimental procedures adopted and the results obtained a brief introduction to the IVIVC, biopharmaceutics classification system (BCS), *in vivo* absorption, IVIVC models, *in vitro* dissolution, IVIVC model development, IVIVC model validation, estimation of the drugs in biological medium and extraction of drugs and metabolites from biological samples would be discussed in detail. The literature on the selected modified release formulations for developing IVIVC, namely, ondansetron hydrochloride and dextromethorphan hydrobromide, would also be reviewed here.

The pharmaceutical industry today is caught between the downward pressure on prices and the increasing cost of successful drug discovery and development. The average cost and time for the development of a new chemical entity is much higher (approximately $500 million and 10–12 years) than those required to develop a Novel Drug Delivery System (NDDS) ($20–50 million and 3–4 years). An existing drug molecule in the form of an NDDS can get a new life, thereby increasing its market value, competitiveness and extending its patent life. Limited formularies, patent expiry with subsequent entry of generic competition and vertical integration have made the entire pharmaceutical industry focus today on designing and developing new and better methods of drug delivery. There has been a significant increase in approvals of NDDS in the past couple of years and this is expected to continue at an impressive rate in the near future[1].

In the past few decades, significant medical advances have been made in the area of drug delivery with the development of novel dosage forms. The delivery of several classes of drugs, however, continues to be a challenge mainly due to their short half-life, poor membrane permeability and associated toxicity in the administered doses. Today we have a better understanding about

the relationship between chemical properties of drugs and their movement in the body. Drug discovery scientists are, therefore, considering the pharmacokinetic properties of agents much earlier in the drug development process.

The rational development of a delivery system is sensible and expensive. Formulation development and optimization involves varying excipient levels, processing methods, identifying, discriminating dissolution methods and subsequent scale up of the final product. As quantitative and qualitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of bio studies is always desirable. In this regard, use of *in vitro* data to predict *in vivo* performance can be considered as the rational development of controlled release formulations.

Recently a regulatory guidance was developed to minimize the need for additional bioavailability studies as part of the formulation design. This guidance referred to as the *In vitro In vivo* Correlation (IVIVC) guidance, was developed by the Food and Drug Administration (FDA) and is based on scientifically sound research.

***In vitro in vivo* correlation**

*In vitro in vivo* correction [IVIVC] has been defined by the Food and Drug Administration [FDA] as a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response[2]. Generally the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is a plasma drug concentration or amount of drug absorbed. The United States Pharmacopoeia [USP] also defines IVIVC as the establishment of a relationship between a biological property or a parameter derived from a biological property produced from a dosage form and a physicochemical property of the same dosage form[3]. Typically, the parameter derived from the biological property is AUC or $C_{max}$, while the physico chemical property is the *in vitro* dissolution profile. A linear

relationship with slope of unity, if possible, is preferred, as the dissolution profile is a representative of the absorption profile[3, 4]. However, non-linear correlation may also be appropriate[2].

IVIVC plays an important role in product development in that it first, serves as a surrogate of *in vivo* and assists in supporting bio waivers; second, supports and/or validates the use of dissolution methods and specifications; and third, assists in quality control during manufacturing and selecting appropriate formulations[2, 5]. The first and main role of establishing IVIVC is to use dissolution test as a surrogate for human studies. The benefit from this is to minimize the number of bioequivalence studies performed during the initial approval process and during the scaling up and post approval changes[2]. Further benefit of the IVIVC is to assist in validating or setting dissolution specifications. This is because the IVIVC includes *in vivo* relevance to *in vitro* dissolution specification. In other words, dissolution specifications are set based on the performance of a bio batch *in vivo*. The general dissolution time point specification is ±10% deviation from the mean dissolutions profile obtained from the bio batch[2]. Bioequivalence between formulations would be expected if the formulation(s) fall within the upper and lower limits of the specification. Dissolution specification setting based on an IVIVC can also be used as a quality control for product performance. However, this quality control may sometimes be more rigorous than the usual control standard since it depends on the product bioavailability.

The use of IVIVC, however, is limited to a certain drug product. It can be used only on that particular formulation. The IVIVC cannot be used across the products, especially drug product with different release mechanisms[2, 6].

IVIVC is usually developed when drug dissolution is a rate-limiting step for the *in vivo* absorption. The absorption and consequently the bioavailability of an oral solid dosage form depend on two main processes, drug dissolution and permeation. Drug dissolution is the process in which the drug is released and available in solution and ready to be absorbed. Physico chemical properties of a drug such as solubility as well as the gastro intestinal environment are the

crucial parameters affecting dissolution. Drug permeability is the ability of the drug to penetrate across a membrane into systemic circulation. The extent of permeation and ultimately absorption also depends upon the physico chemical properties of the drug and blood perfusion[7]. The complete penetration of a highly permeable drug occurs in a short time. Thus, the only factor governing drug absorption is drug release and/or dissolution from the dosage form. *In vitro* drug dissolution can be used as a surrogate for the *in vivo* absorption. Contrary the dissolution rate of immediate release drug products is very rapid. The rate of absorption is likely to be a function of gastric emptying rate or the intestinal permeability. In this case, the IVIVC may not be obtained[6].

Previous IVIVC studies have been reported for various drugs[8-19]. The studies were conducted both in animal such as rat, rabbit, dog and human. Most of the studies focused on the development of a level B and level C correlations. The level B is a correlation in which it compares the mean *in vivo* dissolution to the mean *in vitro* dissolution. The level C correlation describes a relationship between amount of drug dissolved at one time point and one pharmacokinetic parameter. The level C is also considered the lowest level of correlation. All level B and C IVIVCs were developed for several purposes in formulation development, example, for selecting the appropriate excipients and optimizing the manufacturing processes, for quality control purposes and for characterizing the release patterns of a newly formulated immediate release (IR) and modified release (MR) products relative to the reference[8-19]. However, current IVIVC studies have focused on the development and validation of a level A correlation. It is a point-to-point relationship between drug release *in vitro* and *in vivo*. Although, a concern of non - linear correlation has been addressed, no formal guidance exists on the non-linear IVIVC controlling drug absorption[1].

In summary, the IVIVC is established to enable the dissolution test to be used as a surrogate for bioequivalency. It is benefit for pharmaceutical manufacturers due to minimizing the time and cost invested in the bioavailability studies. In addition, IVIVC is normally expected for highly

permeable drugs or drugs under dissolution rate-limiting conditions. This statement is further supported by the regulatory biopharmaceutical drug classification, which anticipates the successful IVIVC for highly permeable drugs[5].

**Biopharmaceutics Classification System (BCS)**

Biopharmaceutics Classification System (BCS) is a fundamental guideline for determining the conditions under which *in vitro in vivo* correlations are expected[5]. It is also used as a tool for developing the *in vitro* dissolution specification[6, 20]. The classification is associated with drug dissolution and absorption model, which identifies the key parameters as a dimensionless number such as the absorption number, the dissolution number and the dose number [5, 6, 20]. The absorption number is the ratio of the mean residence time to mean absorption time. The dissolution number is the ratio of mean residence time to mean dissolution time. The dose number is the mass divided by an uptake volume of 250 ml and drug's solubility. The mean residence time is the average of the residence time in the stomach, small intestine and the colon. The fraction of dose absorbed can be predicted based on these three parameters. For example, absorption number 10 means that the permeation across the intestinal membrane is 10 times faster than the transit through the small intestine indicating 100% drug absorbed.

In the BCS, a drug is classified in one of the four class based solely on its solubility and intestinal permeability[20]: high solubility/high permeability (Class I), low solubility/high permeability (Class II), high solubility/low permeability (Class III) and low solubility/low permeability (Class IV). Class I drugs such as metoprolol exhibit high absorption number and high dissolution number. The rate-limiting step to drug absorption is drug dissolution or gastric emptying rate if dissolution is very rapid. Class II drugs such as phenytoin has a high absorption number but low dissolution number. *In vivo* drug dissolution is a rate-limiting step for absorption (except at very high dose number). The absorption for Class II drugs is usually slower than Class I and occurs over a long period of time. IVIVC is usually expected for Class I and II drugs. For

Class III drugs, permeability is the rate-controlling drug absorption. Furthermore, Class III drugs exhibit a high variability of rate and extent of drug of drug absorbed. Since the dissolution is rapid, the variation is due to alteration of gastro intestinal (GI) physiological properties and membrane permeation rather than dosage form factors. Class IV drugs are low solubility and low permeability drugs. Drugs that fall in this class exhibit a lot of problems for effective oral administration. Drug example for class III and IV is cimetidine and chlorothiazide, respectively.

In general, a high soluble drug is characterized based on the largest dosage strength soluble in 250 ml or less of water over a pH range of 1 - 8. In addition, if the extent of drug absorption is greater than 90% given that the drug is stable in the gastro intestinal environment; it will be considered as a high permeable drug[6].

***In vitro* dissolution**

The purpose of the *in vitro* dissolution studies in the early stage of drug development is to select the optimum formulation, evaluate the active ingredient with excipient and assess any minor change for drug products. However, for the IVIVC perspective, dissolution is proposed to be a surrogate of drug bioavailability. Thus, a more rigorous dissolution standard may be necessary for the *in vivo* waiver[6].

Generally, a dissolution methodology, which is able to discriminate between the study formulations and which best, reflects the *in vitro* behavior would be selected. Four basic types of dissolution apparatus such as rotating basket, paddle method, reciprocating cylinder and flow through cell, are specified by the USP and recommended on the FDA guidance especially, for modified release dosage form[21]. Other dissolution methodologies may be used, however, the above four are preferred, especially the basket and paddle. It is also recommended to start with the basket or paddle method prior to using the others[6].

The *in vitro* dissolution release of a formulation can be modified to facilitate the correlation development. Changing dissolution testing conditions

such as the stirring speed, choice of apparatus, pH of the medium and temperature may alter the dissolution profile. As previously described, appropriate dissolution testing conditions should be selected so that the formulation behaves in the same manner as the *in vivo* dissolution. The appropriate dissolution testing conditions should also discriminate between different formulations that possess different release patterns. A common dissolution medium is water. Others are simulated gastric fluid (pH 1.2), or intestinal fluid (pH 6.8 or 7.4) without enzyme and buffers with a pH range of 4.5 to 7.5[21]. For sparingly water-soluble drugs, use of surfactants in the dissolution medium is recommended[7]. A simple aqueous dissolution media is also recommended for BCS class I drug as this type of drug exhibits lack of influence of dissolution medium properties[22]. Water and simulated gastric fluid are the default mediums for most of the class I drug. A typical medium volume is 500 to 1000ml. The normal test duration for immediate release is 15 to 60 minutes with a single time point. For example, BCS class I recommends 15 minutes. Additionally, two time points may be required for the BCS class II at 15 minutes and the other time at which 85% if the drug is dissolved[7]. In Contrast, *in vitro* dissolution tests for a modified release dosage form require at least three time points to characterize the drug release. The first sampling time 1 - 2 h or 20-30% drug release is chosen to check dose-dumping potential. The intermediate time point has to be around 50% drug release in order to define the *in vitro* release profile. The last time point is to define essentially complete drug release[3, 21]. The dissolution limit should be at least 80% drug release. Further justification as well as 24 h test duration are required if the percent drug release is less than 80[21]. Once the discriminatory system is established, dissolution testing conditions should be fixed for all formulation tested for development of the correlation[2]. A dissolution profile of percentage or fraction of drug dissolved versus time can be determined.

The similarity of the dissolution profiles in particular dissolution testing conditions is evaluated using the similarity factor ($f_2$ metric) defined by equation[23, 24] which is as follows:

$$f_2 = 50 \log \left\{ \left[ 1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} X\ 100 \right\}$$

where $R_t$ and $T_t$ are the cumulative percentage dissolved at time point t for reference and test products, respectively and n is the number of pool points. The $f_2$ equation is a logarithmic transformation of the sum of squares of the difference between test and reference profiles[23]. The results are values between 0 and 100. The value of $f_2$ is 100 when the test and reference profiles are identical and approaches zero as the dissimilarity increases. An average difference of 10% at all time points results in the $f_2$ value of 50. The $f_2$ value between 50 and 100, therefore, suggests the similarity between two dissolution profiles[25]. This equation is only applicable in comparing profiles in which the average difference between R and T is less than 100. If this average difference is greater than 100, the equation will yield a negative number[23]. Normalization of the data is required to compare values in which the difference is not between 1 and 100.

***In vivo* evaluation**

The FDA requires *in vivo* bioavailability studies to be conducted for a New Drug Application (NDA). Bioavailability studies are normally performed in young healthy male adult volunteers under some restrictive conditions such as fasting, non-smoking and no intake of other medications. The drug is usually given in a crossover fashion with a washout period of at least half-life of five. The bioavailability study can be assessed via plasma or urine data using the following parameters:

- Area under the plasma time curve (AUC), or the cumulative amount of drug excreted in urine ($D_u^{\infty}$)
- Maximum concentration ($C_{max}$), or rate of drug excretion in urine ($dD_u \backslash dt$)
- Time of maximum concentration ($T_{max}$)

Several approaches can be employed for determining the *in vivo* absorption. Wagner-Nelson, Loo-Riegelman and numerical de convolution are such methods[23,24]. Wagner Nelson and Loo Riegelman are both model-dependent methods in which the former is used for a one-compartment model and the latter is for multi-compartment system. The Wagner Nelson method is less complicated than the Loo Riegelman as there is no requirement for intravenous data[25]. However, misinterpretation on the terminal phase of the plasma profile may be possible in the occurrence of flip-flop phenomenon in which the rate of absorption is slower than the rate of elimination. De convolution is a numerical method used to estimate the time course of drug input using a mathematical model based on the convolution integral[2].

**Levels of *in vitro in vivo* correlation**

IVIVC is classified by the USP into three levels: A, B and C, depending upon the degree of quality. Level A correlation is the highest level of correlation achievable. It is a 1:1 correlation representing the relationship between *in vitro* dissolution and the *in vivo* absorption rate of a drug from the dosage form[3]. In this level, the *in vitro* dissolution profile for the dosage form is superimposed on the *in vivo* dissolution profile. The advantage of Level A is that a point-to-point correlation is developed. In this case the *in vitro* dissolution, thus, can be used as: (a) a surrogate for the *in vivo* performance, (b) a quality control procedure, which is predictive of product performance, (c) a justification for the extreme *in vitro* quality control standards (by a convolution or deconvolution procedure) and (d) a waiver for additional human studies under the minority changes as specified in the scale up and post approval change immediate release (SUPAC-IR) and scale up and post approval change modified release (SUPAC-MR) guidances[3].

Level A can be developed on both deconvolution and convolution-based methods. Deconvolution based approach (specified in the IVIVC guidance) is a two-stage procedure. The first stage is to determine the time course of *in vivo* absorption rate. The next stage is to develop a correlation between *in vitro* dissolution and *in vivo* absorption rates[2,5]. Convolution-based method, however,

is a one-stage procedure in which IVIVC model directly relates the *in vitro* release profile to the plasma time course[5]. This stage does not require the determination of *in vivo* absorption rate.

Level B compares the mean *in vitro* dissolution time ($MDT_{vitro}$) to the mean *in vivo* residence time ($MRT_{vivo}$) or the mean *in vivo* dissolution time ($MDT_{vivo}$). These parameters are determined by statistic moment analysis[4, 7, 24, 26, 27]. The Level B correlation does not fully describe the curve as does Level A, since it does not uniquely reflect the actual *in vivo* plasma level curve which is a number of different *in vivo* curves can produce the same MRT[4]. Thus, it is less useful level of correlation.

Level C is a single point comparison of the amount of drug dissolved at dissolution time point to one pharmacokinetic parameter (e.g., $C_{max}$, AUC, and $T_{max}$). This is a weak correlation since it does not reflect the plasma or dissolution profiles[3]. The other two levels, which have not been described in the USP but have been addressed in the FDA-IVIVC guidance, are multiple Level C and Level D. Multiple Level C is a correlation involving one or several pharmacokinetic parameters to the amount of drug dissolved at various time points[2]. Its correlation is more meaningful than that of Level C as several time points are considered. Level D is a rank order analysis. In this level, formulation composition or manufacturing variables is related to *in vitro* dissolution data or *in vivo* variables. It is not a formal correlation but serves as an aid in the development of a formulation or processing procedure.

**Development and validation of the *in vitro in vivo* correlation**

**IVIVC Model development**

Linear regression analysis was used to examine the relationship between percentage of the drug dissolved and the percentage of drug absorbed. The percentage of the drug unabsorbed was calculated from the percentage absorbed. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. The dissolution rate constants were determined from % released versus the square root of time.

Linear regression analysis was applied to the *in vitro-in vivo* correlation plots and the coefficient of correlation ($r^2$), slope and intercept values were calculated. Level A correlation was estimated by a two-stage procedure, deconvolution followed by comparison of the percentage drug absorbed to the percentage drug dissolved.

**IVIVC Model validation**

The objective of any mathematical predictive tool is to successfully predict the outcome (*in vivo* profile) with a given model and test condition (*in vitro* profile). Integral to the model development exercise is model validation, which can be accomplished using data from the formulations used to build the model (internal validation) or using data obtained from a different (new) formulation (external validation). While internal validation serves the purpose of providing basis for the acceptability of the model, external validation is superior and affords greater "confidence" in the model.

**Internal validation**

The predictability of the IVIVC was examined by using the mean *in vitro* dissolution data and mean *in vivo* pharmacokinetics of the selected modified release formulations. The mean *in vitro* dissolution rate constants was correlated with the mean absorption rate constants for the modified release formulations. These two data points, along with the zero-zero intercept were used to calculate the expected absorption rate constants.

The prediction of plasma concentration was accomplished using the following curve fitting equation:

$y = \text{Const.} \times (\text{Dose}) \times k_a / k_a - k_{el} (e^{-kelt} - e^{-kat})$

where, y = predicted plasma concentration (ng/ml); Const. = the constant representing F / Vd (where F is the fraction absorbed and Vd is the volume of distribution); $k_a$= absorption rate constant; $k_{el}$= overall elimination rate constant.

To further assess the predictability and the validity of the correlations, the observed and IVIVC model-predicted $C_{max}$ and AUC values for formulation

are determined. The percent prediction errors for $C_{max}$ and AUC were calculated as follows:

$$\% PE_{Cmax} = \left( \frac{C_{max}(obs) - C_{max}(pred)}{C_{max}(obs)} \right) X\ 100$$

$$\% PE_{AUC} = \left( \frac{AUC(obs) - AUC(pred)}{AUC(obs)} \right) X\ 100$$

where % PE is the percent prediction error, $C_{max}$ (obs) & $C_{max}$ (pred) are the observed and IVIVC model-predicted maximum plasma concentrations, respectively; and AUC (obs) & AUC (pred) are the observed and IVIVC model-predicted AUC for the plasma concentration profiles, respectively.

The criteria set in the FDA guidance on IVIVC for level A are as follows: For $C_{max}$ and AUC, the mean absolute % PE should not exceed 10% and the prediction error for individual formulations should not exceed 15%.

**External Validation**

For establishing external predictability, the exposure parameters for a new formulation are predicted using its *in vitro* dissolution profile, the IVIVC model and the predicted parameters are compared to the observed parameters. The prediction errors are computed as for the internal validation. For $C_{max}$ and AUC, the prediction errors for the external validation formulation should not exceed 10%. A prediction error of 10% to 20% indicates inconclusive predictability and illustrates the need for further study using additional data sets. For drugs with narrow therapeutic index, external validation is required despite acceptable internal validation, whereas internal validation is usually sufficient with non-narrow therapeutic index drugs.

**Estimation of the drugs in biological medium**

Methods of measuring drugs in biological media are increasingly important problems related to the following studies and are highly dependent on biopharmaceutical analytical methodology;

- Bioavailability and bioequivalence studies
- New drug development
- Clinical pharmacokinetics
- Research in basic biomedical and pharmaceutical sciences

The most common samples obtained for biopharmaceutical analysis are blood and urine. Faeces are also utilized, especially if the drug or metabolite is poorly absorbed or extensively excreted in the bile. Other media that can be utilized include saliva, breath and tissue.

The choice of sampling media is determined largely by the nature of the drug study. Whole blood is usually collected by venipuncture with either a hypodermic syringe or a vacutainers apparatus. The volume of blood collected at any one sampling time is usually limited to 5 to 15 ml (depending on the assay sensitivity and the total number of samples taken for a given study). If the blood is allowed to clot and is centrifuged, about 30 to 50% of the original volume is collected as serum (upper level). Generally plasma is preferred because of its greater yield from blood. The greater the yield, the greater the amount of drug and the fewer the problems with sensitivity. Blood, serum or plasma samples can be utilized for drug studies and may require protein denaturation steps before further manipulation.

Detection of a drug or its metabolite in biological media is usually complicated by the matrix. Because of this, various types of clean up procedures involving techniques such as solvent extraction and chromatography are

employed to effectively separate drug components from endogenous biological material. The ultimate sensitivity and selectivity of the assay method may be limited by the efficiency of the clean up methodology.

Separation or isolation of drugs and metabolites from biological samples is performed in order to partially purify the sample. In this manner, an analyst can obtain the selectivity and sensitivity needed to detect a particular compound and can do so with minimum interference from components of the more complex biological matrix. The number of steps in a separation procedure should be kept to a minimum to prevent loss of drug or metabolite. Sometimes, the separation steps are preceded by a sample pretreatment.

In order to avoid decomposition or other potential chemical changes in the drugs to be analyzed, biological samples should be frozen immediately upon collection and thawed before analysis. When drugs are susceptible to plasma esterases, the addition of esterase inhibitors such as sodium fluoride to blood samples immediately after collection helps to prevent drug decomposition.

In most cases, preliminary treatment of a sample is needed before proceeding to the measurement step. Drug analyses are required in samples as diverse as plasma, urine, faeces, saliva, bile, sweat and seminal fluid. Each of these samples has its own set of factors that must be considered before an appropriate pretreatment method can be selected. Such factors as texture and chemical composition of the sample, degree of drug-protein binding, chemical stability of the drug and types of interferences can affect the final measurement step.

Biological materials such as plasma, faeces and saliva contain significant quantities of protein that can bind a drug. The drug may have to be freed from protein before further manipulation. Protein denaturation is important because the presence of proteins, lipids, salts and other endogenous materials in the samples can cause rapid deterioration of HPLC columns and also interfere the

assay. Protein denaturation procedures include the use of tungstic acid, ammonium sulfate, heat, alcohol, trichloro acetic acid and perchloric acid.

**Extraction of drugs and metabolites from biological samples**

After pre treating biological material, the next step usually is the extraction of the drugs from the biological matrix. All separation procedures use one or more treatments of matrix-containing solute with some fluid. If the components are a liquid (extracting solvents) and a solid (e.g., lyophilized faeces), it is an example of liquid-solid extraction. If the extraction involves two liquid phases, it is an example of liquid-liquid extraction.

Liquid-solid extractions occur between a solid phase and a liquid phase. Either phase may initially contain the drug substance. Among the solids that have been used successfully in the extraction (usually via adsorption) of drugs from liquid samples are XAD-2 resin, charcoal, alumina, silica gel and aluminum silicate. Liquid-solid extraction is often particularly suitable for polar compounds that would otherwise tend to remain in the aqueous phase. The method could also be useful for amphoteric compounds that cannot be extracted easily from water.

The liquid solid extraction method provides a convenient isolation procedure for blood samples, thus avoiding solvent extraction, protein precipitation, drug losses and emulsion formulation. It is possible, however, that strong drug-protein binding could prevent sufficient adsorption of the drug to resin.

Liquid-liquid extraction is probably the most widely used technique because it can remove a drug or metabolite from larger concentrations of endogenous materials that might interfere with the final analytical determination and also this technique is simple, rapid and has a relatively small cost factor per sample.

Partitioning or distribution of a drug between two possible liquid phases can be expressed in terms of a partition or distribution coefficient. A partition coefficient is a constant only for a particular solute, temperature and pair of solvents used. By knowing the partition coefficient for the extracted drug and the absolute volumes of the two phases to be utilized, the quantity of the drug extracted after a single extraction can be obtained. In multiple extraction methodology, the original biological sample is extracted several times with fresh volumes of organic solvent until maximum possible drug is obtained. As the combined extracts now contain the total extracted drug, it is desirable to calculate the number of extractions necessary to achieve maximum extraction.

Factors that influence partition coefficient and hence recovery of drugs in liquid-liquid extraction are the choice of the solvent, pH and ionic strength of the aqueous phase. It is generally accepted that diethyl ether and chloroform are the solvents of choice for acidic and basic drugs, respectively, especially when the identity of the drugs in the samples is unknown. Chemically neutral drugs are extracted into either solvent depending on their relative partition tendencies.

The presence of metabolites or more than one drug in a biological sample usually demands a more sophisticated separation for their measurement especially, when two or more drugs are of similar physical and chemical nature.

Chromatography is a separation technique that is based on differing affinities of a mixture of solutes between at least two phases. The result is a physical separation of the mixture into its various components. The affinities or interactions can be classified in terms of a solute adhering to the surface of a polar solid (adsorption), a solute dissolving in a liquid (partition) and a solute passing through or impeded by a porous substance based on its molecular size (exclusion).

Most of the drugs in biological samples can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy,

precision, ease of automation and eliminates tedious extraction and isolation procedures.

There are different modes of separation in HPLC. They are normal phase mode, reverse phase mode, reverse phase ion pair chromatography, ion exchange chromatography, affinity chromatography and size exclusion chromatography (gel permeation and gel filtration chromatography).

Methods for analyzing drugs in biological samples can be developed, provided one has knowledge about the nature of the sample, namely, its molecular weight, polarity, ionic character and the solubility parameter. An exact recipe for HPLC, however, cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. In general, one begins with reverse phase chromatography, when the compounds are hydrophilic in nature with many polar groups and are water soluble.

The organic phase concentration required for the mobile phase can be estimated by gradient elution method. For aqueous sample mixtures, the best way to start is with gradient reverse phase chromatography. Gradient can be started with 5 - 10 % organic phase in the mobile phase and the organic phase concentration (acetonitrile or methanol) can be increased up to 100 % within 20-30 min. Separation can be optimized by changing the initial mobile phase composition and the slope of gradient according to the chromatogram obtained from preliminary run. The initial mobile phase composition can be estimated on the basis of where the compounds of interest were eluted, namely, at what mobile phase composition.

Elution of drug molecules can be altered by changing the polarity of the mobile phase. The elution strength of a mobile phase depends upon its polarity, the stronger the polarity, higher is the elution. Ionic samples (acidic or basic)

can be separated, if they are present in undissociated form. Dissociation of ionic samples may be suppressed by proper selection of pH.

The pH of the mobile phase has to be selected in such a way that the compounds are not ionized. If the retention times are too short, the decrease of the organic phase concentration in the mobile phase can be in steps of 5 %. If the retention times are too long, an increase in 5 % steps of the organic phase concentration is needed.

Whenever acidic or basic samples are to be separated it is strongly advisable to control mobile phase pH by adding a buffer and the pH of the buffer should be adjusted before adding organic phase. The buffer selected for a particular separation should be used to control pH over the range of pKa ± 1.0. The buffer should transmit light at or below 220 nm so as to allow low UV detection.

Optimization can be started only after a reasonable chromatogram has been obtained. A reasonable chromatogram means that all the compounds are detected by more or less symmetrical peaks on the chromatogram. By a slight change of the mobile phase composition, the shifting of the peaks can be expected. From a few experimental measurements, the position of the peaks can be predicted within the range of investigated changes. An optimised chromatogram is the one in which all the peaks are symmetrical and are well separated in less run time.

## 2. AIM AND OBJECTIVES

Establishing a correlation between the *in vitro* dissolution profile of a Modified Release (MR) formulations and the *in vivo* plasma concentration profiles have been of great interest for a number of years. Modified release (MR) of drugs in the gastrointestinal (GI) tract following oral administration is the intended rate-limiting factor in the absorption process. It is, therefore, desirable to use *in vitro* data to predict *in vivo* bioavailability parameters for the rational development and evaluation process for extended release dosage forms.

The ultimate goal of an *in vitro–in vivo* correlation (IVIVC) should be to establish a meaningful relationship between *in vivo* behavior of a dosage form and *in vitro* performance of the same, which would allow *in vitro* data to be used as a surrogate for *in vivo* behavior. A meaningful IVIVC for extended release dosage forms would be of benefit as a surrogate for bioequivalence studies which might typically be required with scale up or minor post-approval changes (SUPACs) in formulation equipment, manufacturing process or in the manufacturing site. A meaningful IVIVC could lead to improved product quality and decreased regulatory burden.

It is well known that *in vitro* dissolution testing is a powerful and useful method for determining product quality. The utility of *in vitro* dissolution as a surrogate for *in vivo* bioavailability is very attractive and has been demonstrated for several products. Furthermore to utilize this dissolution test, the IVIVC must be predictive of *in vivo* performance of the product. Levels A, B, C and multiple level C correlations have been described in the US Food and Drug Administration (FDA) IVIVC guidance. The most useful of these is a level A correlation, which is described as a point-to-point correlation, in which the *in vivo* percentage absorbed curve is compared to *in vitro* percentage dissolved curve. Generally, these correlations are linear and are considered most informative and very useful from a regulatory view point. The FDA guidance describes the methods of evaluation of prediction error internally and/or

externally. Internal validation refers to how well IVIVC model describes the data used to develop the correlation. External validation determines how well the IVIVC model describes data that was not used in the development of the model.

Numerous IVIVC studies of modified release formulations have been previously reported. There are no reports, however, of such studies for the drugs of Ondansetron hydrochloride and Dextromethorphan hydrobromide. The purpose of this study was, therefore, to develop IVIVCs for the selected modified release formulations of these drug candidates. The validity of the correlation was proposed to be established through the internal and external predictability.

## 3. REVIEW OF LITERATURE

Several investigations have been carried out in the past on IVIVC and method development for analyzing drugs in biological fluids. A survey of literature was carried out in such investigations. In what follows, some of the important investigations are discussed.

S. Dutta and coworkers[28] have reported once-a-day extended - release dosage form of Divalproex Sodium III: development and validation of a Level A *in vitro* - *in vivo* correlation (IVIVC). Defining a quantitative and reliable relationship between *in vitro* drug release and *in vivo* absorption is highly desired for rational development, optimization and evaluation of controlled-release dosage forms and manufacturing process. During the development of a once-daily extended-release (ER) tablet of divalproex sodium, a predictive *in vitro* drug release method was designed and statistically evaluated using three formulations with varying release rates. In order to establish an internally and externally validated Level A IVIVC, a total of five different ER formulations of divalproex sodium were used to evaluate a linear IVIVC model based on the *in vitro* test method. For internal validation, a single-dose four-way crossover study (N=16) was performed using fast-, medium- and slow-releasing ER formulations and a 12 h IV infusion of valproic acid as reference. To validate the IVIVC externally, a second three-way crossover study (N=36) was performed using slightly-fast-, medium- and slightly-slow-releasing ER formulations. The *in vivo* absorption–time profile was inferred by deconvolution of the observed plasma concentration–time profiles against the unit disposition function (UDF). A linear IVIVC model was established in which the *in vivo* absorption was expressed as a function of *in vitro* drug release. Plasma profiles of ER formulations were estimated via convolution of *in vitro* release profiles with the UDF. Successful internal and external validations of the model were demonstrated by individual and average absolute percent prediction errors of </=9% for both $C_{max}$ and $AUC_{\infty}$. In conclusion, a Level A IVIVC describing the

entire time-course of plasma concentrations was developed and validated, both internally and externally, for ER formulations of divalproex sodium.

S. Hayes and coworkers[29] have reported on interpretation and optimization of the dissolution specifications for a modified release product with an *In Vivo–In Vitro* Correlation (IVIVC). Almost invariably, the *in vitro* dissolution test is interpreted in terms of bioequivalence. The literature that describes methods for setting *in vitro* dissolution specifications is reviewed. The most common interpretation of these specifications is a deterministic one, that is, those batches passing the dissolution specifications would be bioequivalent with the reference if tested *in vivo* and those failing the dissolution specifications would not be bioequivalent if tested *in vivo*. Due to random variation, the deterministic interpretation is not appropriate. Instead, one need to consider the conditional probability that a batch that has passed the *in vitro* dissolution test would demonstrate bio equivalence if tested *in vivo*, and that a batch known to have failed the *in vitro* dissolution test would demonstrate bio inequivalence if tested *in vivo*. One way to estimate these probabilities is by means of a simulated experiment in which the production and testing (*in vivo* and *in vitro*) of a large number of batches is computer simulated. Such a simulation can only be performed if the relationship between the *in vitro* dissolution characteristics and the *in vivo* performance of the product has been modeled. These models are generally referred to as *in vivo–in vitro* correlations (IVIVC). The results of one such experiment are described. The above-mentioned conditional probabilities are shown to depend on the choice of dissolution specifications. This result leads to the notion of optimal dissolution specifications. However, both of the conditional probabilities cannot be maximized simultaneously. The probability of making a correct decision on the basis of the *in vitro* dissolution test is introduced as a possible optimality criterion. This probability is a linear combination of the two conditional probabilities of interest. Using this criterion, the optimal dissolution specifications can be found by searching over the multi dimensional space defined by the half width of each interval used in the specifications to find the

combination that maximizes this probability. This process is demonstrated using the Nelder-Mead search routine. The choice of dissolution specifications has profound implications for the routine production of the product because if the specifications were very narrow the probability of a batch passing would be low, resulting in a low hit rate. The same computer program used to perform the simulation experiment can be used to estimate the hit rate. Furthermore, it can be used to explore the magnitude of changes required in the parameters describing the test product (particularly variability) to increase a low hit rate to an acceptable level.

H. Kortejarvi and coworkers[30] have reported Level A *In Vitro - In Vivo* Correlation (IVIVC) model with Bayesian Approach to formulation Series. *In vitro - in vivo* correlation (IVIVC) models for formulation series are useful in drug development, but the current models are limited by their inability to include data variability in the predictions. Goal was to develop a level A IVIVC model that provides predictions with probabilities. The Bayesian approach was used to describe uncertainty related to the model and the data. Three bioavailability studies of levosimendan were used to develop IVIVC model. Dissolution was tested at pH 5.8 with basket. The IVIVC model with Bayesian approach consisted of prior and observed data. All observed data were fitted to the one-compartment model together with prior data. Probability distributions of pharmacokinetic parameters and concentration time profiles were obtained. To test the external predictability of IVIVC model, only dissolution data of formulations E and F were used. The external predictability was good. The possibility to utilize all observed data when constructing IVIVC model, can be considered as a major strength of Bayesian approach. For levosimendan capsule data traditional IVIVC model was not predictable. The usefulness of IVIVC model with Bayesian approach was shown with our data, but the same approach can be used more widely for formulation optimization and for dissolution based biowaivers.

A. Savaser and coworkers[31] have reported preparation and *in vitro* evaluation of sustained release tablet formulations of diclofenac sodium. The

effects of formulation variables on the release profile of diclofenac sodium (DS) from hydroxyl propyl methyl cellulose (HPMC) and chitosan matrix tablets were studied. DS tablets were prepared by wet granulation and direct compression methods and different ratios of HPMC and chitosan were used. Physical properties of the prepared tablets and targeted commercial sustained release (SR) tablet and the drug release were studied in tablets that were placed in 0.1M HCl for 1 h and phosphate buffer solution was added to reach pH value of 7.5. *In vitro* studies showed that 20% HPMC contained SR formulation with direct (dry) compression method is the optimum formulation due to its better targeting profile in terms of release. This formulation also exhibited the best-fitted formulation into the zero order kinetics. The precision and accuracy of the analytical method were also checked. The repeatability and reproducibility of the method were also determined.

V.R.Uppoor[32] has reported regulatory perspectives on *in vitro* (dissolution) / *in vivo* (bioavailability) correlations. *In vitro* dissolution has been extensively used as a quality control tool for solid oral dosage forms. In several cases, however, it is not known whether one can predict the *in vivo* performance of these products from *in vitro* dissolution data. In an effort to minimize unnecessary human testing, investigations of *in vitro* / *in vivo* correlations (IVIVC) between *in vitro* dissolution and *in vivo* bioavailability are increasingly becoming an integral part of extended release (ER) drug product development. This increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry. Because of the scientific interest and the associated utility of IVIVC as a valuable tool, the US Food and Drug Administration has published Guidance in September 1997, entitled extended release oral dosage forms: development, evaluation and application of i*n vitro* / *in vivo* Correlations. A predictive IVIVC enables *in vitro* dissolution to serve as a surrogate for *in vivo* bioequivalence testing. IVIVCs can be used in place of bio studies that may otherwise be required to demonstrate bioequivalence, when certain pre approval and post approval changes are made in formulation, equipment, manufacturing process or in the manufacturing site. IVIVC

development could lead to improved product quality (more meaningful dissolution specifications) and decreased regulatory burden (reduced bio study requirements). FDA Guidance which deals with the development, evaluation methods, criteria and applications of IVIVCs. From a regulatory point of view, the applications of IVIVC to grant bio waivers and to set dissolution specifications for ER oral dosage forms are presented. Additionally, since the principles of IVIVC are considered to be similar for non-oral dosage forms, the guidance for oral extended release products may be applied for non-oral products as well. While the principles are likely to be the same, it is an interesting challenge to look at appropriate methods for dissolution testing and for development of *in vitro* / *in vivo* correlations for products such as injectable depot formulations.

J. Emami and coworkers[33] have reported *in vitro* – *in vivo* evaluation of sustained - release lithium carbonate (LC) matrix tablets: influence of hydrophilic matrix materials. Sustained-release matrix tablets were therefore developed using different types and ratios of polymers including carbomer (CP), Na carboxy methyl cellulose (Na CMC) and hydroxyl propyl methyl cellulose (HPMC), to assess the release profiles and *in vivo* performance of the formulations. The tablets were prepared by either direct compression (DC) or wet granulation (WG). In the DC method, 69% (450 mg) LC, 5, 10 or 15% CP or Na CMC (of total tablet weight), lactose and /or Avicel (to maintain constant tablet weight) were mixed and directly compressed. In the WG method, 450 mg LC and 10, 20, or 30% HPMC were granulated with Eudragit S100 solution, dried and then compressed to formulate the tablets. *In vitro* and *in vivo*, newly formulated sustained-release LC tablets were compared with sustained-release commercial tablets. *In vivo* studies were conducted in nine healthy subjects in a cross-over design, with a 3x3 latin square sequence and pharmacokinetic parameters were estimated using classical methods.

V.H. Sunesen and coworkers[34] have reported *in vivo in vitro* correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with bio relevant dissolution media. The purpose of the study was to design

dissolution tests that were able to distinguish between the behavior of danazol under fasted and fed conditions, by using bio relevant media. *In vitro* dissolution of 100 mg danazol capsules was performed using the flow-through dissolution method. Flow rates were 8, 16 or 32 ml/min, corresponding to total volumes dissolution medium of 960, 1920 and 3840 ml, respectively. The media used contained bile salt and phospholipid levels relevant for either fasted or fed conditions *in vivo*. Crude and inexpensive bile components, porcine bile extract and soybean phospholipids, were used as the bile source. The effect of adding different concentrations and molar ratios of mono glycerides and fatty acids to the fed state media was investigated. *In vivo* release profiles under fasted and fed conditions were obtained from a previous study by deconvolution. In the fasted state, the physiologically most relevant correlation with *in vivo* results was achieved with a medium containing 6.3mM bile salts and 1.25mM phospholipids (8 ml/min). A medium containing 18.8mM bile salts, 3.75mM phospholipids, 4.0mM mono glycerides and 30mM fatty acids (8 ml/min) gave the closest correlation with fed state *in vivo* results. By using the flow-through dissolution method it was possible to obtain correlations with *in vivo* release of danazol under fasted and fed conditions. Both hydrodynamics and medium composition were important for the dissolution of danazol. In the fed state an IVIVC could only be obtained by including mono glycerides and fatty acids in the medium.

J.B. Dressman and coworkers[35] have reported *in vitro – in vivo* correlations for lipophilic, poorly water-soluble drugs. Although several routes of administration can be considered for new drug entities, the most popular remains the oral route. To predict the *in vivo* performance of a drug after oral administration from *in vivo* data, it is essential that the limiting factor to absorption can be modelled *in vitro*. In the case of BCS class II drugs dissolution is rate-limiting to absorption, so the use of bio relevant dissolution tests can be used to predict differences in bioavailability among different formulations and dosing conditions. To achieve an a priori correlation, the composition, volume and hydrodynamics of the contents in the gastrointestinal lumen following

administration of the dosage form must be accurately simulated. Four media have been chosen/developed to model composition of the gastric and intestinal contents before and after meal intake. These are SGF, milk, FASSIF and FESSIF, which model fasted and fed state conditions in the stomach and small intestine respectively. Using these media, excellent correlations have been obtained with the following poorly soluble drugs: danazol, ketoconazole, atovaquone and troglitazone. In all cases, fed vs. fasted state effects can be predicted from dissolution data and where several formulations were available for testing, dissolution tests could also be used to determine which would have the best *in vivo* performance.

F. Langenbucher[36] has reported on handling of computational *in vitro*/*in vivo* correlation problems by Microsoft Excel: IV. Generalized matrix analysis of linear compartment systems. A linear system comprising n compartments is completely defined by the rate constants between any of the compartments and the initial condition in which compartment(s) the drug is present at the beginning. The generalized solution is the time profiles of drug amount in each compartment, described by poly exponential equations. Based on standard matrix operations, an Excel worksheet computes the rate constants and the coefficients, finally the full time profiles for a specified range of time values.

R.Y. Cheung and coworkers[37] have reported a new approach to the *in vivo* and *in vitro* investigation of drug release from loco regionally delivered microspheres. The purpose of this work was to determine the *in vivo* release profile of doxorubicin (Dox) delivered loco regionally by dextran-based microspheres (MS) and to develop an *in vitro* method for predicting *in vivo* drug release from MS— I*n Vitro*–I*n Vivo* correlation (IVIVC). For the determination of *in vivo* Dox release, drug-loaded MS were placed into hollow fibers (HF) and implanted subcutaneously into C3H mice. Samples were retrieved at various times following implantation, MS removed from HF and the amount of Dox remaining determined via ultraviolet/visible (UV/Vis) spectrophotometry. Various *in vitro* systems were designed and investigated for their ability to link *in vivo* and *in vitro* release profiles, including an open system (e.g. a column)

with continuous flow of release medium at different flow rates and closed systems (e.g. a cuvette) using different release media and conditions. About 34% of loaded Dox was released from MS *in vivo* at 48 h. Only an incremental release was observed over the ensuing 72 h. The release kinetics of Dox from MS using three of the investigated *in vitro* systems, column system and HF immersed in a buffer solution or growth medium gave release profiles that were highly correlated with the *in vivo* release profile ($r^2$>9). The relationships, both linear and non-linear, suggest that Level A IVIVC models can be developed for Dox release from loco regionally delivered MS using specially designed release systems.

B. De Spiegeleer and coworkers[38] have reported dissolution stability and IVIVC investigation of a buccal tablet. Using a recently developed bending point criterion to describe certain dissolution profiles, a physical stability screening study of a muco adhesive buccal tablet was performed in order to obtain a fast and useful *in vitro* testing system that allows the assessment of the physical stability of new formulations in a much faster way compared to the standard formal stability tests in which it takes months before conclusions can be drawn. The obtained dissolution results at normal, accelerated and stress conditions are correlated with each other, resulting in a rapid test system to evaluate the physical stability of the tablets. Last, a significant *in vivo in vitro* correlation (IVIVC) was established between the *in vivo* residence time in the buccal cavity and the *in vitro* bending point obtained from the dissolution data. For this particular case study, it is concluded that around 50% of the *in vivo* variability of the residence time in the mouth is explained by the *in vitro* bending point.

K.D.Vlugt-Wensink and coworkers[39] have reported pre clinical and clinical *in vitro in vivo* correlation of an hGH dextran microsphere formulation. The purpose was to investigate the *in vitro in vivo* correlation of a sustained release formulation for human growth hormone (hGH) based on hydroxyl ethyl methacrylated dextran (dex-HEMA) microspheres in Pit-1 deficient Snell dwarf mice and in healthy human volunteers. A hGH-loaded microsphere formulation

was developed and tested in Snell dwarf mice (pharmacodynamic study) and in healthy human volunteers (pharmacokinetic study). Single subcutaneous administration of the microspheres in mice resulted in a good correlation between hGH released *in vitro* and *in vivo* effects for the hGH-loaded microsphere formulation similar to daily injected hGH indicating a retained bioactivity. Testing the microspheres in healthy volunteers showed an increase (over 7–8 days) in hGH serum concentrations (peak concentrations: 1–2.5 ng/ml). A good *in vitro in vivo* correlation was obtained between the measured and calculated (from *in vitro* release data) hGH serum concentrations. Moreover, an increased serum concentration of biomarkers (insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3) was found again indicating that bioactive hGH was released from the microspheres. Good *in vitro in vivo* correlations were obtained for hGH-loaded dex-HEMA microspheres, which is an important advantage in predicting the effect of the controlled drug delivery product in clinical situations.

Y.Wang and coworker[40] have reported on bias in the Wagner–Nelson estimate of the fraction of drug absorbed. The purpose was to examine and quantify bias in the Wagner-Nelson estimate of the fraction of drug absorbed resulting from the estimation error of the elimination rate constant (k), measurement error of the drug concentration and the truncation error in the area under the curve. Bias in the Wagner-Nelson estimate was derived as a function of post-dosing time (t), k, ratio of absorption rate constant to k (r) and the coefficient of variation for estimates of k (CVk), or CV% for the observed concentration, by assuming a one-compartment model and using an independent estimate of k. The derived functions were used for evaluating the bias with r = 0.5, 3, or 6; k = 0.1 or 0.2; CV, = 0.2 or 0.4; and CV, =0.2 or 0.4; for t = 0 to 30 or 60. Estimation error of k resulted in an upward bias in the Wagner-Nelson estimate that could lead to the estimate of the fraction absorbed being greater than unity. The bias resulting from the estimation error of k inflates the fraction of absorption versus time profiles mainly in the early post-dosing period. The magnitude of the bias in the Wagner-Nelson estimate resulting

from estimation error of k was mainly determined by CV. The bias in the Wagner-Nelson estimate resulting from estimation error in k can be dramatically reduced by use of the mean of several independent estimates of k, as in studies for development of an *in vivo* - *in vitro* correlation. The truncation error in the area under the curve can introduce a negative bias in the Wagner-Nelson estimate. This can partially offset the bias resulting from estimation error of k in the early post-dosing period. Measurement error of concentration does not introduce bias in the Wagner-Nelson estimate. Estimation error of k results in an upward bias in the Wagner-Nelson estimate, mainly in the early drug absorption phase. The truncation error in AUC can result in a downward bias, which may partially offset the upward bias due to estimation error of k in the early absorption phase. Measurement error of concentration does not introduce bias. The joint effect of estimation error of k and truncation error in AUC can result in a non-monotonic fraction-of-drug-absorbed-versus-time profile. However, only estimation error of k can lead to the Wagner-Nelson estimate of fraction of drug absorbed greater than unity.

G. Torrado and coworkers[41] have reported on correlation of *in vitro* and *in vivo* acetaminophen availability from albumin micro aggregates oral modified release formulations. The aim of this study was to develop albumin micro aggregated oral formulations for controlled drug release and to reveal the possible influence of the release site on drug absorption. Acetaminophen was chosen as the model drug, which is included in the Class 1 group of the Biopharmaceutics Classification System (BCS). Albumin micro aggregates were formulated into tablets to obtain different drug release rates: Immediate Release (IR) tablets, multi particulate systems with an intermediate release rate and matrix systems showing slow release rate. The properties of the products were initially tested via dissolution studies and then via bioavailability studies in healthy volunteers. Controlled release albumin micro aggregated acetaminophen formulations for oral administration were obtained. The extent of drug absorption was comparable for all formulations, suggesting that the differences found in saliva concentration and urine cumulative profiles could be

attributed merely to differences in drug release kinetics, as confirmed by the *in vitro–in vivo* correlation study. Therefore, it can be concluded that extended release of acetaminophen does not influence its absorption via intestinal heterogeneity.

J.T. Dalton and coworkers[42] have reported predictive ability of Level A *in vitro–in vivo* correlation for ring cap controlled release acetaminophen tablets. The goal of this study was to establish and validate an *in vitro-in vivo* correlation (IVIVC) for two sustained-release formulations (a matrix tablet and a ring cap banded matrix tablet) containing 750 mg of acetaminophen. The *in vitro* dissolution and *in vivo* disposition of these formulations were examined by using a USP type III dissolution apparatus and a single-dose, three-way, crossover study that included an immediate-release acetaminophen dosage form, respectively. An IVIVC was established by using the mean fraction dissolved (FD) and mean fraction absorbed (FA) and used to simulate the plasma concentration-time profile of acetaminophen after administration of the matrix tablet (internal validation) and ring cap banded matrix tablet (external validation). A statistically significant relationship ($r^2 = 0.997$, $P < 0.001$) existed between the FD and FA for matrix tablets and was best described by the equation (FA) = 0.984 x (FD) + 0.0133. The percent predictions errors in $C_{max}$ and AUCL were <10% when predicting the plasma concentration-time profiles for the two formulations, validating the internal and external predictability of the IVIVC. The data (i) show that *in vitro* dissolution data are a good predictor of *in vivo* fraction absorbed for acetaminophen, (ii) support the general use of *in vitro* dissolution data for readily soluble and readily absorbed drugs, (iii) suggest that acetaminophen may serve as a model drug for evaluating novel sustained-release delivery systems, and (iv) provide a tangible example of the limitations of current methods for predicting and validating IVIVC.

H. Mahayni and coworkers[43] have reported evaluation of "external" predictability of an *in vitro-in vivo* correlation for an extended-release formulation containing metoprolol tartrate. The purpose of this study was to examine the external predictability of an *in vitro-in vivo* correlation (IVIVC) for a

metoprolol hydrophilic matrix extended-release formulation, with an acceptable internal predictability, in the presence of a range of formulation/manufacturing changes. In addition, this report evaluated the predictability of the IVIVC for another formulation of metoprolol tartrate differing in its release mechanism. Study 1 examined the scale up of a matrix extended-release tablet from a 3 kg small batch (I) to a 50 kg large batch (II). The second study examined the influence of scale and processing changes [3 kg small batch with fluid bed granulation and drying (III); 80 kg large batch with high shear granulation and microwave drying (IV) and a formulation with an alternate release mechanism formulated as a multi particulate capsule (V)]. *In vitro* dissolution of all formulations (I-V) was conducted with a USP apparatus I at pH 6.8 and 150 rpm. Subjects received the metoprolol formulations and serial blood samples were collected over 48 h and analyzed by a validated HPLC assay using fluorescence detection. A previously developed IVIVC was used to predict plasma profiles. Prediction errors (PE) were <10% for $C_{max}$ and area under the curve (AUC) of concentration versus time for I, II and IV. The $C_{max}$ for III was slightly under estimated (11.7%); however, the PE of the AUC was <10%. Formulation V displayed a PE for $C_{max}$ > 20% and an AUC within 5% of observed values. The low PEs for $C_{max}$ and AUC observed for I - IV strongly suggest that the metoprolol IVIVC is externally valid, predictive of alternate processing methods (IV), scale-up (II, III) and allows the *in vitro* dissolution data to be used as a surrogate for validation studies. However, the lack of predictability for V supports the contention that IVIVCs are formulation specific.

P. Veng Pedersen and coworkers[44] have reported carbamazepine level - A *in vivo* - *in vitro* correlation (IVIVC): a scaled convolution based predictive approach. A method is presented for prediction of the systemic drug concentration profile from *in vitro* release/dissolution data for a drug formulation. The method is demonstrated using four different tablet formulations containing 200 mg carbamazepine (CZM), each administered in a four way cross-over manner to 20 human subjects, with 15 blood samples drawn to determine the resulting concentration profile. Amount versus time

dissolution data were obtained by a 75 rpm paddle method for each formulation. Differentiation, with respect to time, of a monotonic quadratic spline fitted to the dissolution data provided the dissolution rate curve. The dissolution curve was through time and magnitude scaling mapped into a drug concentration curve via a convolution by a single exponential and the estimated unit impulse response function. The method was tested by cross-validation, where the *in vivo* concentration profiles for each formulation were predicted based on correlation parameters determined from *in vivo-in vitro* data from the remaining three formulations. The mean prediction error (MPE), defined as the mean value of 100% x (observed-predicted)/observed was calculated for all 240 cross-validation predictions. The mean values of MPE were in the range of 10-36% (average 22%) with standard deviations (S.D.s) in the range of 9-33% (average 13%), indicating a good prediction performance of the proposed *in vivo - in vitro* correlation (IVIVC) method.

G. Balan and coworkers[45] have reported *In Vitro - In Vivo* Correlation (IVIVC) models for metformin after administration of modified-release (MR) oral dosage forms to healthy human volunteers. The objective of the current study was to develop and evaluate the internal predictability for level C and A *In Vitro-In Vivo* Correlation (IVIVC) models for prototype modified-release (MR) dosage forms of metformin. *In vitro* dissolution data for metformin were collected for 22 h using a USP II (paddle) method. *In vivo* plasma concentration data were obtained from 8 healthy volunteers after administration of immediate-release (IR) and MR dosage forms of metformin. Linear level C IVIVC models were developed using dissolution data at 2.0 and 4.0 h and *in vitro* mean dissolution time (MDT). A deconvolution-based level A model was attempted through a correlation of percent *in vivo* input obtained through deconvolution and percent *in vitro* dissolution obtained experimentally. Further, basic and extended convolution level A IVIVC models were attempted for metformin. Internal predictability for the IVIVC models was assessed by comparing observed and predicted values for $C_{max}$ and $AUC_{\infty}$. The results suggest that highly predictive level C models with prediction errors (%PE) of

<5% could be developed. Mean percent *in vivo* input for metformin was incomplete from all formulations and did not exceed 35% of dose. The deconvolution-based level A models for all MR formulations were curvilinear. However, a unique IVIVC model applicable to all MR formulations could not be developed using the deconvolution approach. The basic convolution level A model, which used *in vitro* dissolution as the *in vivo* input, had %PE values as high as 103%. Using an extended convolution approach, which modeled the absorption of metformin using a Hill function, a level A IVIVC model with %PE as low as 11% was developed. The work indicates that level C and A IVIVC models with good internal predictability may be developed for a permeability- and absorption window-limited drug such as metformin.

N. Sirisuth and coworkers[46] have reported development and validation of a non-linear IVIVC model for a diltiazem extended release formulation. *In vitro* dissolution of diltiazem capsules was examined using the following methods: USP Apparatus II (paddle) at 100 rpm and USP Apparatus III at 30 dpm. Seven healthy subjects received three diltiazem formulations (90 mg): slow (S), moderate (M), fast (F) releasing and an oral solution (90 mg). Serial blood samples were collected over 48 h and analyzed by a validated HPLC assay using ultraviolet detection. The $f_2$ metric (similarity factor) was used to analyze the dissolution data. Linear and non-linear (quadratic, cubic, and sigmoid functions) correlation models were developed using pooled fraction dissolved (FRD) and fraction absorbed (FRA) data from various combinations of the formulations. Predicted diltiazem concentrations were obtained by convolution of the *in vivo* dissolution rates. Prediction errors were estimated for $C_{max}$ and AUC to determine the validity of the correlation. Apparatus II using purified water was found to be the most discriminating dissolution method. Significant intersubject (CV%>50) was observed for $C_{max}$ and AUC. The quadratic M/F IVIVC model provided a significant relationship between FRD and FRA when using either two or three of the formulations. An average percent prediction error for $C_{max}$ and AUC for all formulations was 12.4% and 9.2%, respectively. The prediction errors observed for $C_{max}$ and AUC suggest

that the predictability of the quadratic IVIVC model is inconclusive, as such, external validation studies are required.

O.A.Lake and coworkers[47] have reported *in vitro/in vivo* correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers. The aim of the study was to select a dissolution test method for carbamazepine (CBZ) immediate release tablets, giving the best *in vitro/in vivo* correlations (IVIVC) and to determine the potential of this method as an estimate for bioequivalence testing. Four 200 mg CBZ products which are sold on the Dutch market, covering the innovator and three generic products were selected. They had been tested in a randomized; four way cross-over bioavailability study in healthy volunteers. Their dissolution rate behaviour *in vitro* was investigated in two dissolution media: (1) 1% sodium lauryl sulphate in water (SLS), in accordance with the United States Pharmacopeia (USP); (2) 0.1 mol/l Hydrochloric acid in water (HC). In the bioavailability study these products had shown no large differences in the extent of absorption ($AUC_{0-\infty}$) but large differences in absorption rate. The products now also showed large differences in dissolution rate *in vitro* in both dissolution media, the rank order being the same as for the absorption rate. It was concluded that the absorption rate *in vivo* depends on the dissolution rate *in vivo*. 'Level C' IVIVC according to the USP were optimized by plotting percentages dissolved on selected time points (D values) or their reciprocals (1/D values), against several pharmacokinetic parameters primarily related to the absorption phase and against $AUC_{0-\infty}$. In this way for each IVIVC the optimum D or 1/D value, was calculated. For both media no meaningful IVIVC were obtained with $AUC_{0-\infty}$, but favourable IVIVC were obtained with the parameters primarily related to the absorption phase. In the bioavailability study indicated above it was found that, among the pharmacokinetic characteristics primarily related to the absorption phase, $C_{max}$ is the most promising in expressing rate of absorption in bioequivalence testing in single dose studies with CBZ immediate release tablets. Consequently, $C_{max}$ was selected for expressing rate of absorption. The most favorable IVIVC were

obtained with D(20) in SLS versus $C_{max}$. From this IVIVC and the requirements for bioequivalence $AUC_{0-\infty}$: 0.8-1.25 and $C_{max}$: 0.75-1.35; 90% confidence interval), a specification for dissolution testing in SLS was calculated as follows: after 20 minutes, 34-99% dissolved. Owing to the fact that the rate of absorption *in vivo* depends on the dissolution rate *in vivo*, it can be concluded that with this specification bioequivalence with respect to both rate of absorption and extent of absorption is ensured. As this specification is comparable with the USP specification: not less than 75% dissolved after 1 h, it is concluded that the USP specification is suitable to ensure bioequivalence of CBZ immediate release tablets.

Natalie and coworkers[48] have reported *in vitro in vivo* correlation with metoprolol extended release tablets using two different releasing formulations: an internal validation evaluation. The objective of this analysis was to develop and validate internally an *In Vitro In Vivo* Correlation (IVIVC) for a hydrophilic matrix extended release metoprolol tablet using a combination of two formulations with different release rates. Three formulations of a hydrophilic matrix extended release tablet were manufactured to release metoprolol at a slow, moderate and fast rate. The *in vitro* dissolution methods utilized USP Apparatus II, pH 6.8 at 150 rpm. Seven healthy subjects received three metoprolol formulations (100 mg): slow, moderate and fast releasing and an oral solution (50 mg). Serial blood samples were collected over 48 h and analyzed by a validated HPLC assay using fluorescence detection. The $f_2$ metric (similarity factor) was used to analyze the dissolution data. Correlation models were developed using pooled fraction dissolved (FRD) and fraction absorbed (FRA) data from various combinations of two formulations (slow/moderate; moderate/fast and slow/fast). Predicted metoprolol concentrations were obtained by convolution of the *in vivo* dissolution rates. Prediction errors were estimated for $C_{max}$ and AUC to determine the validity of the correlation. An average percent prediction error for $C_{max}$ and AUC for all formulations of lessthan 12% was found for all IVIVC models. The relatively low prediction errors for $C_{max}$ and AUC observed strongly suggest that the metoprolol IVIVC

models with two formulations used in development are valid. Previous IVIVC with all three formulations was also found to be valid. The relatively low prediction error indicates that the correlations are predictive when using two or three formulations and allows the associated dissolution data to be used as a surrogate for bioavailability studies.

S. Takka and coworkers[49] have reported development and validation of an *in vitro-in vivo* correlation for buspirone hydrochloride extended release tablets. The aim of this study was to develop an *In Vitro-In Vivo* Correlation (IVIVC) for two buspirone hydrochloride extended release formulations and to compare their plasma concentrations over time with the commercially available immediate release (IR) tablets. *In vitro* release rate data were obtained for each formulation using the USP Apparatus 2, paddle stirrer at 50 and 100 rpm in 0.1 M HCl and pH 6.8 phosphate buffer. A three-way cross over study in 18 healthy subjects studied a 30 mg "Fast" (12 h) and 30 mg "Slow" (24 h) formulation of buspirone hydrochloride given once a day, and 2x15 mg immediate release tablets dosed at a 12 h interval. The similarity factor ($f_2$) was used to analyze the dissolution data. A linear correlation model was developed using percent absorbed data and percent dissolved data from the two formulations. Predicted buspirone hydrochloride concentrations were obtained by use of a curve fitting equation for the immediate release data to determine the volume of distribution and fraction absorbed constants. Prediction errors were estimated for $C_{max}$ and area under the curve (AUC) to determine the validity of the correlation. pH 6.8 at 50 rpm was found to be the most discriminating dissolution method. Linear regression analyses of the mean percentage of dose absorbed versus the mean *in vitro* release resulted in a significant correlation ($r^2>0.95$) for the two formulations. An average percent prediction error for $C_{max}$ was -0.16%, but was 16.1%, for the AUCs of the two formulations.

Korteja and coworkers[50] have reported development of level A, B and C *in vitro-in vivo* correlations for modified-release levosimendan capsules. The aim of this study was to investigate the possibility of developing different levels of

correlation between *in vitro* release and *in vivo* absorption rate for four modified-release levosimendan capsule formulations. Differences and similarities in the *in vitro* dissolution curves were compared with pharmacokinetic parameters describing absorption rate. Formulations F, G, H and I differed in the amounts of the delaying excipients alginic acid and HPMC. *In vitro* release rate was studied by the USP basket method using the following conditions: pH 5.8 or 7.4 and a rotation speed of 50 or 100 rpm. *In vivo* bioavailability was tested in nine healthy male volunteers and the fractions absorbed were calculated by the Wagner-Nelson method. Dissolution conditions pH 5.8 and a rotation speed of 100 rpm predicted best the similarities and differences in absorption rates among different formulations and levels C and B correlation coefficients were 0.85 and 0.97, respectively. For formulation H level A correlation (r=0.997) was found when *in vitro* lag time was 0.2 h and time scale factor 1.9. This study indicated that dissolution tests developed can be used as a surrogate for human bioequivalence studies, for development processes of final commercial products, to ensure batch to batch bioequivalence and in the future in possible scale-up and post approval change cases for modified-release levosimendan formulation H.

1) Research work was conducted at the Department of Pharmaceutical Analysis, J.S.S. College of Pharmacy, Ootacamund – 643001, Tamilnadu, India.
2) Part of Research work was conducted at ISO 9001-2000 certified bioequivalence centre at the Centre For Advanced Drug Research And Testing (CADRAT), J.S.S. College of Pharmacy, Ootacamund – 643001, Tamilnadu, India

## 5. SCOPE AND PLAN OF WORK

Use of *in vitro* drug release data to predict *in vivo* bioavailability parameters are desirable for rational development and evaluation of modified release (MR) dosage forms. Development and applications of predictive mathematical relationships between *in vitro* drug release and *in vivo* drug absorption data, reduces the need for *in vivo* bioequivalence tests to document unchanged quality and performance of MR products that undergo certain pre and post-approval changes.

The development of a correlation is based on the scientific principles associated with mathematical modeling, statistical evaluation and numerical deconvolution. The development and validation of an IVIVC is based on the ability of fraction of the drug absorbed versus fraction of the drug-dissolved relationship of various formulations.

The aim of *In Vitro - In Vivo* Correlation (IVIVC) is thus to enable the dissolution testing of modified release formulations poses many challenges. These challenges include developing and validating the test method, ensuring that the method is appropriately discriminatory and addressing the potential of an IVIVC.

A suitable dissolution method is capable of distinguishing the performance of formulations with different release rates, *in vitro* and *in vivo*, is an important tool in product development. IVIVC facilitates the process of such method development. Depending on the nature of the correlation further changes to the dissolution method can be made. When the discriminatory *in vitro* method is validated, further formulation development can be relied on the *in vitro* dissolution only.

Bioavailability and bioequivalence studies involve mathematical analysis of plasma level versus time curves which permits the estimation of half life, absorption rate, excretion rate, extent of absorption and other constants that are useful in describing the fate of given drug in an organism. It should be noted,

however, that neither bioavailability nor bioequivalence data could be generated without analytical methodology to accurately measure drugs in biological fluids.

For the estimation of the drugs present in the biological fluid, HPLC method is considered to be more suitable since it is a powerful and rugged method and also extremely specific, linear, precise, accurate, sensitive and rapid.

The present study, therefore, aims to develop and validate IVIVC of selected modified release formulations containing ondansetron hydrochloride and dextromethorphan hydrobromide. At present there are no IVIVC studies and no sustained release formulations for these drug candidates have been reported in India. The present IVIVC studies, however, focus on the development and validation of a level A correlation.

**Plan of Work**

The project was carried out in the following stages:

**Stage I Preformulation studies**

1. Determination of physical properties of the drugs such as physical nature (amorphous or crystalline), solubility, melting point, etc.

2. Drug compatibility studies were performed by infra red (IR) spectral matching and differential scanning calorimeter (DSC) approach.

**Stage II Development of oral controlled / sustained drug delivery systems**

1. Single unit development of matrix tablets by wet granulation formulation and characterization of granules for
    - Angle of repose,
    - Loose bulk density,
    - Tapped bulk density,
    - Compressibility index and
    - Drug content.
2. Compression of the formulated granules into tablets and evaluation of

the tablets as per the pharmacopeial specifications for

- Average weight and weight variation,
- Thickness,
- Diameter,
- Drug content and content uniformity,
- Hardness,
- Friability,
- *In vitro* drug release behavior and comparison of the release with the marketed conventional dosage forms and
- Optimization of certain process and formulation variables on the physicochemical properties and *in vitro* drug release profile of the formulated tablets.

**Stage III Stability studies as per the ICH guidelines**

Selected batches from the above studies were subjected to stability studies at the following different temperature and humidity conditions as prescribed by the International Conference on harmonization (ICH).

- 25°C with 60 % RH
- 40°C with 75 % RH

Samples were withdrawn at different time intervals and evaluated for their physicochemical parameters and *in vitro* drug release behavior.

**Stage IV Bioavailability study design and data handling**

A randomized, three treatment, three period, three sequence, single dose, crossover bioavailability study was conducted for the innovator conventional formulation and test modified formulation in six healthy, adult, male, human subjects under fasting conditions.

**Stage V Development HPLC methods for the estimation of selected drugs in plasma samples**

Chromatographic conditions like

- Selection of wavelength,

- Selection of initial separation conditions,
- Nature of the stationary phase,
- Nature of the mobile phase (pH, peak modifier, ratio and flow rate) and
- Selection of internal standard were optimized.

**Stage VI Validating the developed method**

Validation parameters such as,

- Accuracy and Precision,
- Linearity and Range,
- Limit of detection (LOD) / Limit of quantitation (LOQ),
- Selectivity / Specificity,
- Robustness / Ruggedness,
- Stability and System suitability of the developed methods were validated.

**Stage VII Pharmacokinetic parameters**

After estimating the selected drugs in human plasma, the following pharmacokinetic parameters were calculated;

- $C_{max}$ Maximum plasma concentration
- $T_{max}$ Time of maximum plasma concentration
- $AUC_{0-t}$ Area under plasma concentrations time curve 0 to 24 h
- $AUC_{0-\infty}$ Area under plasma concentrations time curve 0 to ∞ h
- $t_{1/2}$ Elimination half-life
- $k_{el}$ Elimination rate constant

**Stage VIII. Development of IVIVC correlations**

After carrying out an *in vivo* and *in vitro* data analysis, IVIVC for the developed MR formulations was validated by internal and external predictability approach.

# 6. MATERIALS AND METHODS

## 1. Reagents and Chemicals used

Acetonitrile, methanol, ortho phosphoric acid, potassium di hydrogen ortho phosphate, trifluoro acetic acid, perchloric acid and triethylamine were supplied by Qualigens Fine Chemicals and S.D. Fine chemicals. Water (HPLC grade) was obtained from Milli-QR system. All the reagents and chemicals used were of HPLC or Analytical grade.

Working standards of ondansetron hydrochloride was purchased from Microchem Services (Bangalore, India) and Dextromethorphan hydrobromide was a gift sample from Divis Laboratories (Hyderabad, India). HPMC (Methocel – K100–CR, apparent viscosity, 2% in water at 20°C is 80,000-12000 cP), carbopol and starch 1500 were gift samples from Colorcon Asia Pvt Ltd (Goa, India). Polyvinyl pyrrolidine (PVP-K-30) was a gift sample from Anshul Agencies (Mumbai, India). Aerosil was purchased from Degussa India Pvt Ltd (Mumbai, India).

### 1.1. Instruments used

i. Sartorius single pan digital balance (R200D & 1702)

ii. Systronics pH meter, μ pH system 361

iii. Shimadzu LC 2010A HT HPLC system with the following configurations;

- Low pressure gradient quaternary pump
- Auto injector
- Multi wavelength UV array detector
- Column oven and Degasser
- Class-VP 6.01 data station

iv. Electrolab dissolution testing apparatus

- USP Type II apparatus TDL-08L

v. Shimadzu 160A UV-VIS spectrophotometer

vi. Shimadzu FT IR 8400S spectrophotometer

vii. Ultra Sonicator

viii. Solid phase Extractor

ix. Analytical column such as,

- VYDAC Monomeric $C_{18}$ (250× 4.6mm, 5µ)
- Princeton SPHER HPLC $C_{18}$ (250× 4.6mm, 5µ)
- Phenomenex Luna $C_{18}$ (250× 4.6mm, 5µ)
- Kromasil $C_{18}$ (250× 4.6mm, 5µ)
- Zorbax $C_8$ (250× 4.6mm, 5µ)
- Hypersil $C_4$ (250× 4.6mm, 5µ)
- Solid phase extraction cartridges used Samprep - SPE Columns $C_{18}$ (50µm, 70A) 100mg/1ml

## 2. Experimental

This chapter describes the experimental details of the preformulation study, tablet manufacture, bio availability study design and data handling, optimization and validation of the bio analytical methods for the estimation of ondansetron hydrochloride and dextromethorphan hydrobromide in human plasma samples, preparation of standard and sample solutions, development of *in vitro* dissolution methods, *in vitro* data analysis, *in vivo* data analysis, statistical analysis of pharmacokinetic data and development and validation of level A *In Vitro - In Vivo* Correlation (IVIVC).

### 2.1. Preformulation Study

Preformulation in the broadest sense encompasses all the activities and studies that are required to convert an active pharmacological substance into a

suitable dosage form. It can be defined as an investigation of the physical and chemical properties of a drug substance alone and also when combined with the excipients. In the present study, therefore, evaluation of granulations, development of *in vitro* dissolution method and the compatibility between the drug and the selected polymer were determined.

### 2.1.1. Evaluation of Granulations

The following parameters were used for the characterization of prepared granules

(1) Flow properties

(2) Granular densities

(3) Percentage of fines

#### 2.1.1.1. Flow Properties

The flow properties are critical for an efficient tableting operation. A good flow of the powder or granulation is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets. In some cases, dry powder has to be pre-granulated to improve their flow properties. During the pre-formulation, the flow ability of the drug and granulation should be studied especially when the anticipated dose of the drug is large.

When a heap of powder is allowed to stand with only the gravitational force acting on it, the angle between the free surface of the static heap and the horizontal plane can achieve a certain maximum value for a given powder. This angle is defined as the static angle of repose and is a common way of explaining flow characteristics of powder granulation. In most pharmaceutical powders and granules, the angle of repose values range from 25-40°, with lower values indicating better flow characteristics.

The angle of repose is defined as the maximum angle possible between the surface of a pile of powder or granules and the horizontal plane.

$$\text{Tan } \theta = h/r$$

where,

h and r are the height and radius of the powder cone

$\theta = \tan^{-1} h/r$

**2.1.1.2. Bulk Density**

The weighed amount of the powder was introduced into a graduated measuring cylinder. The cylinder was fixed on the bulk density apparatus and the timer knob was set for 100 tapping. The volume occupied by the powder was noted. Further, another 50 tapping may be continued and final volume was noted. This final volume is bulk volume. Bulk density is defined mathematically as given below

Bulk density = mass of powder / Bulk volume.

Bulkiness = 1 / Bulk density

**2.1.1.3. Percentage of fines**

Percentage of fines was determined by passing the granules through sieves 22 and 40. The particles which pass through # 40 are considered fines.

**2.2. Differential Scanning Calorimeter**

The possibility of drug–excipient interaction was investigated by differential scanning calorimetry. The DSC thermograms of pure drugs ondansetron hydrochloride, Dextromethorphan hydrobromide respectively, individual excipients and drug– excipient mixtures were recorded.

**2.3.Compatibility studies**

Infrared spectral matching approach was employed to detect any possible chemical interaction between ondansetron hydrochloride, dextromethorphan hydrobromide and the polymer. Physical mixtures of the drug and the polymer (1:1) were mixed with 400 mg of potassium bromide (IR grade). About 100 mg of the mixture was taken and compressed to form a transparent pellet in a hydraulic press at 15 tonnes pressure. The samples were scanned from 4000 to 400 $cm^{-1}$ in a Shimadzu FT IR spectrophotometer.

Similarly, the IR spectra of ondansetron hydrochloride, dextromethorphan hydrobromide and the polymer were also recorded. Physical appearance of the samples and appearance /disappearance of peaks in the spectra were observed to assess any possible physical and chemical interactions.

**3. Tablet Manufacture**

**3.1.Development of Ondansetron hydrochloride Sustained Release (SR) tablets**

Ondansetron hydrochloride SR tablets were prepared by the wet granulation method (Figure 1). All the composition, with the exception of magnesium stearate and aerosil were thoroughly mixed in a tumbling mixer for 5 min and wetted in a mortar with isopropyl alcohol. The wet mass was sieved (16 mesh) and granules were dried at 60°C for 2 h. The dried granules were sieved (22 mesh) and these granules were lubricated with a mixture of magnesium stearate and aerosil (2:1). The ondansetron tablets were prepared using an electrically operated punching machine. Compression was performed after granulation process with a single punch press applying a compression force of a 9 KN (preliminary work) or 12 KN (experimental design), equipped with a 6 mm concave punch. For the preliminary work, batches of 100 tablets were prepared. Each batch of experimental design consisted of 100 tablets (drug content in the tablet was 8 mg). Three batches were prepared for each formulation and the compositions of different batches of ondansetron hydrochloride SR tablets are given in Table 1. The compressed tablets were evaluated for average weight and weight variation, thickness, diameter, drug content & content uniformity, hardness, friability, disintegration and *In vitro* drug release.

**3.2. Development of Dextromethorphan hydrobromide Sustained Release (SR) tablets**

Dextromethorphan hydrobromide SR tablets were prepared by the wet granulation method (Figure 1). All the composition, with the exception of magnesium stearate and aerosil were thoroughly mixed in a tumbling mixer for 5 min and wetted in a mortar with isopropyl alcohol. The wet mass was sieved

(16 mesh) and granules were dried at 60°C for 2 h. The dried granules were sieved (22 mesh) and these granules were lubricated with a mixture of magnesium stearate and aerosil (2:1). The dextromethorphan hydrobromide tablets were prepared using an electrically operated punching machine. Compression was performed after granulation process with a single punch press applying a compression force of a 9 KN (preliminary work) or 12 KN (experimental design), equipped with a 8 mm concave punch. For the preliminary work, batches of 100 tablets were prepared. Each batch of experimental design consisted of 100 tablets (drug content in the tablet was 60 mg). Three batches were prepared for each formulation and the compositions of different batches of dextromethorphan hydrobromide SR tablets are given in Table 2. The compressed tablets were evaluated for average weight & weight variation, thickness, diameter, drug content and content uniformity, hardness, friability, disintegration and *In vitro* drug release.

### 3.3. Evaluation of Tablets

The prepared tablets were evaluated for the following properties:

1. Thickness
2. Hardness
3. Friability

#### 3.3.1. Hardness

The hardness of a tablet is indication of its strength. It is tested by measuring the force required to break the tablet across the diameter. The force is measured in kg and the hardness of about 4 kg is considered to be satisfactory for uncoated tablets. Monsanto hardness tester is used for this purpose. The hardness of 10 tablets was measured and the average hardness was calculated.

#### 3.3.2. Friability Test

Friability is the loss of weight of tablets in the container, due to removal of fine particles from their surfaces. Friability test is carried out to assess the ability of the tablet to withstand abrasion in packing, handling and transport. Roche friability tester was used for finding out the friability of the tablet. A number of tablets (10) were weighed accurately and placed in the chamber of the apparatus. After 100 rotations, the tablets were taken out from the apparatus, re-dusted and weighed. The loss in weight indicates the friability of the tablets. A maximum friability of 1% is acceptable for tablets as per Indian Pharmacopoeia (IP). Percentage friability was determined by using the formula given below:

$$\% \text{ friability} = (W_1 - W_2 / W_1) \times 100$$

where $W_1$ = weight of tablets before test

$W_2$ = weight of tablets after test

#### 3.3.3. *In vitro* drug release

Dissolution was performed using an Electro lab – Tablet dissolution Tester, USP XXIII Model. The media used was 0.1N Hcl at pH 2.0 and a volume

of 700 ml for the first 2 h after which 200 ml of 0.2 M sodium phosphate tribasic, was added to give a final pH of 7.5 and maintained at 37°C. Dissolution tests were performed on six tablets and the amount of drug released was analyzed by HPLC.

### 3.3.4. Stability studies as per the ICH guidelines

Developed SR tablets were packed in High Density Poly Ethylene (HDPE) containers and were subjected to stability studies at the following different temperature and humidity conditions as prescribed by the International Conference on Harmonization (ICH)[51].

- 25°C with 60 % RH
- 40°C with 75 % RH

Samples were withdrawn at 1, 2, 3 and 6 months intervals and evaluated for their physical properties and *in vitro* drug release.

## 4.0. Bioavailability studies

Bioavailability studies of the optimized formulations were carried out in cross over design in healthy human volunteers between the developed formulations and the conventional dosage form. The protocol of the study was submitted to the Institutional Human Ethical Committee and the approval for conducting the same was obtained and prior consent of the volunteers participated in the study was taken.

A randomized, three-treatment, three-period, three-sequence, single dose, cross over bioavailability studies were carried out in healthy human volunteers between the developed sustained release (SR) formulation and the marketed conventional immediate release (IR) formulation to prove the safety and efficacy of the developed SR formulation. A reproducible analytical technique was developed for the estimation of the drugs in the plasma samples. Various

pharmacokinetic parameters such as $C_{max}$, $T_{max}$, $t_{1/2}$, $k_{el}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were estimated.

The subjects for the bioavailability study were selected from the panel of volunteers enrolled with the Centre of Bioequivalence, J.S.S. College of Pharmacy, Ootacamund. Volunteers were screened for inclusion in the study 7 days prior to the commencement of the study. Restrictions on admission into the study were based on the following safety considerations:

- Healthy males, 22-30 years of age,
- Not more than ±15 % from ideal weight for subject's height and elbow breadth,
- General good health as determined by medical history and physical examination within 30 days prior to the start of the study (without a history of clinically significant organ - system disorders or ongoing infectious diseases, history of benign prostatic hypertrophy, prostate infections or urinary retention, history of asthma and drug allergy history of peripheral neuropathy, history of alcohol abuse or drug addiction requiring treatment within the last 12 months),
- No prescription drugs within 14 days or Over The Counter (OTC) preparations, herbal remedies or nutritional supplements (excluding vitamins) within 7 days prior to drug administration and
- Subjects with systolic blood pressure 90 - 140 mm Hg, diastolic pressure 50 - 90 mm Hg and pulse rate within 50 - 100 bpm.

On the basis of this preliminary screening, 24 volunteers were selected and their liver function, renal function and haematological parameters such as hemoglobin content, RBC and WBC counts, blood sugar, cholesterol, bilirubin and ECG were examined by standard clinical and biochemical investigations. No grapefruit juice or grapefruit containing products for at least 72 h and caffeine or xanthine consumption for at least 12 h prior to drug administration

was allowed in each period. No concomitant medication (other than the study drug) was allowed during the study phase. Volunteers were also instructed to refrain from consuming alcohol, smoking or other stimulant drinks during this period.

Prior to the commencement of the study, each subject was provided with an information sheet giving details of the investigational drugs, procedure, potential risk involved and a written consent was obtained. They were instructed that they are free to withdraw their consent and to discontinue their participation in the study at any time without prejudice.

All the volunteers were made to assemble in the bioequivalence Centre, 12 h prior to the initiation of the study. After overnight fasting, the volunteers were given code numbers and allocated to the treatment in accordance with the randomized code. Their pulse rates and blood pressures were recorded and a sterile intravenous cannula (size 20) introduced with strict aseptic precautions for blood collection. Volunteers received either test or reference formulations (Table 3) according to their code numbers with 240 ml of water. The order of treatment administration was randomized in three sequences (ABC, BCA, and CAB) in blocks of three.

Blood samples (4 ml) were collected using disposable syringes in pre-heparinised centrifugal tubes at 0 (before drug administration), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h post dosing. The samples were centrifuged at 3500 rpm for 10 min to separate plasma. They were transferred into air tight containers and stored at deep freeze condition (-70$^{o}$C) until starting of the analysis. A similar procedure adopting cross over design in drug treatment was repeated after 7 days of wash out period.

The study was monitored by a physician and a clinical pharmacologist. In addition, a staff nurse and a technician were present throughout the study for blood collection and plasma separation. The blood pressure and pulse rate were measured at 0.5, 1, 3, 6, 12 and 24 h post dosing. The volunteers were

monitored for abnormal symptoms during the study period and for one week, after the study period and if noticed, the details were entered in the case report sheets and tabulated at the end of the study.

Standard breakfast was provided after 3 h post dosing. Subjects were instructed to eat their entire breakfast in 30 min. Lunch and dinner, consisting of caffeine-free, xanthine-free, grapefruit-free foods and beverages were served after 7 and 12 h post dosing during the in house portion of the study.

All the plasma samples were extracted using solid phase extraction (SPE) and their drug levels were quantified using HPLC techniques.

Pharmacokinetic parameters namely, $C_{max}$, $T_{max}$ and $k_{el}$ were determined for individual drug treatments from the observed plasma concentration-time data. AUC were calculated by trapezoidal rule from time zero to the last observed concentration.

Blood samples were collected using disposable syringes in pre-heparinised centrifugal tubes at different time intervals. The samples were centrifuged at 3500 rpm for 10 min to separate plasma. They were transferred into air tight containers and stored at deep freeze condition until starting of analysis. A similar procedure adopting cross over design in drug treatment was repeated after 7 days of wash out period.

All the plasma samples were extracted by solid phase extraction (SPE) method and their drug levels were quantified using HPLC technique.

Pharmacokinetic parameters such as peak plasma concentration ($C_{max}$), Time to peak concentration ($T_{max}$), Area under the plasma concentration - time curve ($AUC_{0-t}$ & $AUC_{0-\infty}$), elimination rate constant ($k_{el}$) and Elimination half-life ($t_{1/2}$) were calculated separately and the blood level data of the reference product and the test products were compared. The ln-transformed values of $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ along with the factors included in this statistical analysis were periods, sequences, treatments and subjects. The factor subject was random and others were fixed. A difference between the treatments was

calculated including the 95% confidence interval of that difference. The design statement indicates that the subjects were tested within the sequences.

## 5.0. Estimation of drugs

### 5.1. Optimization of chromatographic conditions for the estimation

Proper selection of the chromatographic method depends upon the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. The drugs selected for the present study are polar in nature and hence either reverse phase or ion pair or ion exchange chromatography can be used. For the present study reverse phase HPLC methods are considered to be more suitable because they are extremely specific, linear, precise, accurate, sensitive and rapid methods.

#### 5.1.1. Selection of detection wavelength for ondansetron hydrochloride and dextromethorphan hydrobromide

10 µg/ml of ondansetron hydrochloride and dextromethorphan hydro bromide were prepared, individually in solvent mixtures of methanol and water (1:1). These solutions were scanned in the UV region of 200 - 400 nm and the UV spectrums were recorded (Figures 2 and 3). From the spectra, detection wavelength 305 and 280 nm was selected for ondansetron hydrochloride and dextromethorphan hydrobromide, respectively.

### 5.1.2. Initial separation conditions

A gradient run was performed for the initial separation. From this the approximate ratio of the organic phase in the buffer solution required to elute the drugs from the column was determined. An aliquot of the standard solution was prepared and chromatogrammed using the following chromatographic conditions;

| | | |
|---|---|---|
| Stationary phase | : | Phenomenex Luna $C_{18}$ Column, (5 µ, 25 cm X 4.6 mm i.d and 5 µ, 10 cm X 4.6 mm i.d) |
| Mobile phase | : | Solvent A : 25 mM Phosphate buffer / 0.5% trifluoro acetic acid<br>Solvent B : Acetonitrile / Methanol |
| Solvent ratio | : | Gradient run, 10 to 100% Solvent B for 20 min |
| Flow rate | : | 1.0 ml/min |
| Sample size | : | 50 µl |
| Temperature | : | Room temperature of $20^0 \pm 1^0$ C |

From the above gradient run, the approximate percentage of acetonitrile or methanol in the phosphate buffer or trifluoro acetic acid buffer required to elute the drugs from the column was determined (Table 4). This ratio was used for subsequent isocratic separation and the chromatograms were recorded.

### 5.1.3. Effect of chromatographic variables

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, solvent strength, addition of peak modifiers, flow rate, solvent ratio and the nature of stationary phase on the peak separation were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor,

resolution and column efficiency were calculated. The conditions that gave the best symmetry and capacity factor were selected for the estimation.

The standard solution of ondansetron hydrochloride and dextromethorphan hydrobromide were chromatogrammed for 20 min using acetonitrile in buffer solutions of different pH ranging from 2.0 to 7.0 as the mobile phase at a flow rate of 1.0 ml/min. It was observed that an increase in pH decreases the retention time of dextromethorphan hydrobromide and increases retention time of ondansetron hydrochloride.

Dextromethorphan hydrochloride peak eluted with void volume at pH range 5.5 to 7.0, whereas ondansetron hydrochloride well retained and symmetrical peaks were obtained. These conditions were, therefore, selected for further studies.

As peak modifier, 0.1 % triethylamine was added separately to the mobile phase to improve the peak shape. Triethylamine (0.5%) in buffer did not improve the peak retention or shape and hence 0.5% triethylamine in acetonitrile was not selected as peak modifier.

**5.1.4. Nature of the stationary phase**

Different reverse phase stationary phases ($C_4$, $C_8$ and $C_{18}$) were used and the chromatograms were recorded. When $C_4$ and $C_8$ columns were used, the retention times of the drugs were reduced.

Based on the retention and peak shape, Princeton SPHER HPLC $C_{18}$ column was selected for ondansetron hydrochloride, whereas, VYDAC Monomeric $C_{18}$ reverse-phase column was selected for dextromethorphan hydrobromide.

Different mobile phases, namely, acetonitrile, methanol and tetrahydrofuran in aqueous phase were used at a flow rate of 1.0 or 0.5 ml/min. The strength of water, acetonitrile, methanol and tetrahydrofuran in reverse phase chromatography were 0.0, 3.2, 2.6 and 4.5, respectively. For the initial

separation conditions, acetonitrile was used. When acetonitrile was substituted by other solvents, the solvents to buffer ratios were calculated using solvent strength. The resulting ratios of the mobile phase were prepared and the drugs were chromatogrammed. These mobile phases gave well retained and symmetrical peaks. Tetrahydrofuran was not selected due to its UV cut off wavelength of 215 nm. Methanol or acetonitrile was used as the mobile phase for further studies.

### 5.1.5. Selection of internal standard

Internal standards may be used along with the standard drugs to minimize the error in the assay due to loss of drugs that occur during extraction procedure. Internal standards were selected on the basis of purity, polarity, solubility and absorption characteristics. The internal standards selected for the present study was Etoricoxib and Losartan potassium for Ondansetron hydro chloride and Dextromethorphan hydrobromide, respectively. These internal standards provide well resolved and symmetrical peaks.

## 5.2. Optimized chromatographic conditions

Based on the above studies, the following chromatographic conditions were selected for the estimation of selected drugs in plasma samples and dissolution samples.

### 5.2.1. Chromatographic Conditions for Ondansetron hydrochloride

| | |
|---|---|
| Stationary phase | : Princeton SPHER $C_{18}$ (250 x 4.6 mm i.d., 5µ) |
| Mobile Phase | : Acetonitrile: 50 mM phosphate buffer of pH 7.0 |
| Mobile phase ratio | : 60:40 % v/v |
| Flow rate | : 1. 0 ml/min |
| Sample volume | : 50 µl using Rheodyne 7725i injector |
| Detection | : 305 nm using SPD-M10A VP Diode Array Detector |
| Data station | : Class VP data station |

Internal Standard : Etoricoxib

**5.2.2. Chromatographic Conditions for Dextromethorphan hydrobromide**

Stationary phase : VYDAC Monomeric $C_{18}$ (250 x 4.6 mm i.d., 5µ)

Mobile Phase : Acetonitrile: 0.5% trifluoro acetic acid

Mobile phase ratio : 40:60 % v/v

Flow rate : 1. 0 ml/min

Sample volume : 50µl using Rheodyne 7725i injector

Detection : 280 nm using SPD-M10A VP Diode Array Detector

Data station : Class VP data station.

Internal Standard : Losartan potassium

**6.0. Preparation of standard and sample solutions**

**6.1.Preparation of standard and sample Ondansetron hydrochloride solutions**

**a. Standard stock solution of Ondansetron hydrochloride**

10 mg of Ondansetron hydrochloride working standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in methanol - water mixture (1:1) and made up to the volume with the same solvent to produce a 1mg/ml of ondansetron hydrochloride. The stock solution was stored in refrigerator at –20 ± $2^0$ C until analysis.

The stock solution was diluted to suitable concentrations for spiking plasma to obtain calibration curve (CC) standards and quality control (QC) samples.

**b. Calibration Curve Standards and Quality Control Samples**

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution using water. Calibration standards for control plasma were prepared by spiking this stock solution to obtain the concentration levels of 0.5, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0 and 50.0 ng/ml in human plasma. Quality control samples were prepared as bulk, at a concentration of 0.5 ng/ml (LLOQ QC), 1.0 ng/ml (LQC), 10.0 ng/ml (MQC) and 40.0 ng/ml (HQC).

These samples were stored below -50°C until use.

**c. Standard stock solution of Etoricoxib (Internal Standard)**

10 mg of etoricoxib internal standard was accurately weighed and transferred into a 10 ml volumetric flask, dissolved in acetonitrile - water mixture (1:1) and made up to the volume with the same solvent to produce a 1mg/ml of etoricoxib. The stock solution was stored in refrigerator at $20 \pm 2^0$ C until analysis.

The stock solution was diluted to suitable concentration (1 µg/ml) with HPLC grade water, prior to use as internal standard.

**d. Plasma samples**

Calibration standards, validation QC samples and healthy volunteer plasma samples were prepared by adding 0.5 ml plasma to Eppendorf tube followed by adding 10.0 µl internal standard solution (1.0 µg/ml). All samples were mixed by vortexer for 30 s. After these procedures, Samprep SPE Column $C_{18}$ (50µm, 70Å) 100mg/1ml solid phase extraction cartridge was conditioned with methanol, water sequentially and sample was loaded. The cartridge was washed with 2.0 ml of water. The drug and internal standard were eluted from the cartridge using 0.5ml of methanol. The resulting solution was used for the analysis.

**6.2. Preparation of standard and sample Dextromethorphan hydrobromide solutions**

**a. Standard stock solution of Dextromethorphan hydrobromide**

10 mg of dextromethorphan hydrobromide working standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in methanol and water mixture (1:1) and made up to the volume with the same solvent to produce a 1mg/ml of dextromethorphan hydrobromide. The stock solution was stored in refrigerator at $-20 \pm 2^0$ C until analysis.

The stock solution was diluted to suitable concentrations for spiking plasma to obtain calibration curve (CC) standards and quality control (QC) samples.

**b. Calibration Curve Standards and Quality Control Samples**

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution using water. Calibration standards for control plasma were prepared by spiking this stock solution to obtain the concentration levels of 110.0, 140.0, 230.0, 590.0, 1000.0, 1400.0, 2100.0, 3000.0 ng/ml in human plasma. Quality control samples were prepared as bulk, at a concentration of 110.0 ng/ml (LLOQ QC), 590.0 ng/ml (LQC), 1400.0 ng/ml (MQC) and 3000.0 ng/ml (HQC).

These samples were stored below -50°C until use.

**c. Standard stock solution of Losartan potassium (Internal Standard)**

10 mg of losartan potassium internal standard was accurately weighed and transferred into a 10 ml volumetric flask, dissolved in acetonitrile and water mixture (1:1) and made up to the volume with the same solvent to produce a 1 mg/ml of losartan potassium. The stock solution was stored in refrigerator at –20 ± $2^0$ C until analysis.

The stock solution was diluted to suitable concentration (100 µg/ml) with HPLC grade water, prior to use as internal standard.

**d. Plasma samples**

Calibration standards, validation QC samples and healthy volunteer plasma samples were prepared by adding 0.5 ml plasma to Eppendorf tube followed by adding 10.0 µl internal standard solution (100.0 µg/ml). All samples were mixed by vortexer for 30 s. After these procedures, Samprep SPE Columns $C_{18}$ (50µm, 70A) 100mg/1ml solid phase extraction cartridge was conditioned with methanol, water sequentially and sample was loaded. The cartridge was washed with 2.0 ml of water. The drug and internal standard were eluted from the cartridge using 0.5ml of mobile phase. The resulting solution was used for the analysis.

## 7.0. Validation of HPLC methods

Validation is a process which involves confirmation or establishment by laboratory studies that a method / procedure / system / analyst can give the required accuracy, precision, sensitivity, ruggedness, etc. In the most basic form, validation of an analytical procedure demonstrates that the procedure developed is suitable for its intended purpose. Validation of the method was carried out after the development of the HPLC methods.

Validation parameters tested were,

1. Selectivity/ Specificity
2. Sensitivity
3. Linearity
4. Precision and Accuracy
    a. Within-batch precision and accuracy
    b. Intra-day precision and accuracy
    c. Between batch / Inter-day precision and accuracy
5. Stabilities
    a. Short Term Stock Dilution Stability
    b. Long Term Stock Solution Stability
    c. Freeze Thaw Stability
    d. Bench Top (BT) Stability
    e. Long-Term (LT) Stability
    f. Autosampler Stability
6. Recovery
7. Ruggedness
8. Robustness

### 7.1. Selectivity/ Specificity

A method is said to be specific when it produces a response only for a single analyte. Method selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences. In order to prove

that the method chosen was specific and selective the following two sets of samples were processed and injected into the HPLC using the extraction procedure.

1. Blank samples from six different lots of biological matrix (plasma containing $K_2$ EDTA as anticoagulant).
2. Samples from the same six lots of biological matrix mentioned in step 1 spiked with the analyte at the lower limit of quantification (LLOQ) of the calibration curve and with the internal standard at the concentration level in the study.

To calculate % interference, the response obtained for each sample in step 1 was compared with the response obtained for each corresponding sample in step 2.

% Interference=(Peak area response of blank/ Peak area response of LLOQ) X 100

**7.2. Sensitivity**

It is expressed as limit of quantitation. It is the lowest amount of analyte in a sample matrix that can be determined.

The lower limit of quantification for ondansetron hydrochloride was 0.5 ng/ml and dextromethorphan hydrobromide was 110.0 ng/ml.

**7.3. Linearity**

Linearity and range of the methods were analyzed by preparing calibration curves using different concentrations of the standard solution containing the internal standard. The calibration curve was plotted using response factor and concentration of the standard solutions.

Linearity was established using four calibration curves over the range of (0.5 to 50.0ng/ml for ondansetron hydrochloride, 110.0 to 3000.0ng/ml for dextromethorphan hydrobromide) using the weighted least square regression analysis.

A calibration curve consisted of

- Aqueous standard at middle concentration level to check retention time of analyte and internal standard.
- Blank sample (matrix sample processed without internal standard and analyte)
- Zero sample (matrix sample processed with internal standard but without analyte)
- Eight non-zero standards covering the expected range. The lowest and highest standards were prepared in duplicates.

### 7.4. Precision and Accuracy

The precision and accuracy of the method was determined by analyzing two batches each consisting of one set of calibration curve with six replicates of quality control samples at four concentration levels [Quality Control samples at the lower limit of quantification (QCLLQ), Low (QCL), Middle (QCM) and High(QCH)].

**Precision**

Precision is expressed as the percentage coefficient of variation (%CV) which is calculated as per the following expression:

% CV = (Standard Deviation /Mean) x 100

*Intra-run Precision*

Intra-run precision was determined by calculating the percentage coefficient of variation (% CV) of the results obtained in the same run.

*Intra-day Precision*

Intra-day precision was determined by calculating the percentage coefficient of variation (% CV) of the results obtained in the same day.

*Inter-day Precision*

Inter-day precision was determined by calculating the percentage coefficient of variation (% CV) of the results obtained over at least two days.

**Accuracy**

Accuracy is reported as % nominal of the analyzed concentration which is calculated as:

% Nominal = (Measured Concentration / Actual Concentration) x 100

*Intra-run Accuracy*

Intra-run accuracy was determined by calculating the percentage nominal of the calculated concentration from the actual values for quality control samples at each concentration level analyzed in a single run and the mean of percentage nominal at each level was reported.

*Intra-day Accuracy*

Intra-day accuracy was determined by calculating the percentage nominal of the calculated concentration from the actual values for quality control samples at each concentration level analyzed in a single day and the mean of percentage nominal at each level was reported.

*Inter-day Accuracy*

Inter-day accuracy was determined by calculating the percentage nominal of the calculated concentration from the actual value for quality control samples at each concentration level analyzed over at least two days and the mean of percentage nominal at each level was reported.

## 7.5. Stock Solution Stability

### 7.5.1. Short Term Stock Dilution Stability

The stability of stock dilutions of analyte and the internal standard was evaluated at room temperature. Aqueous stock dilution of the analyte and the internal standard were prepared. One portion of the stock dilution was placed in the refrigerator between 2-8°C, while the other portion was placed at room temperature for 24 h. Stock dilution stored at room temperature (stability samples) was compared with refrigerated stock dilutions considered as 'comparison samples'. Six replicate injections of the above solutions were made. Acceptance Criteria: % stability should be within 90 to 110% or the % change should be ± 10%.

% Stability = (Mean response of stability samples / Mean response of comparison samples) × 100

% Change = 100 – (Mean response of stability samples / Mean response of comparison samples × 100)

### 7.5.2. Long Term Stock Solution Stability

The stability of the stock solution when stored in the refrigerator for a given period of time was determined. Stock solutions of the analyte and internal standard were prepared and stored in the refrigerator between 2 - 8°C for 7 days (stability stock). The stock solution stabilities of the analyte and the internal standard were determined with a comparison stock solution, which was prepared freshly. Five replicate injections of the above solutions were made. The response of comparison samples were corrected by multiplying with correction factor to nullify the difference between the measured weights or the dilutions made.

Correlation factor (CF) = Concentration of comparison stock / Concentration of stability stock

% Stability = (Mean response of stability samples / Mean response of comparison samples) × CF x 100

% Change = 100 – (Mean response of stability samples / Mean response of comparison samples) × CF × 100

### 7.5.3. Freeze Thaw Stability

This test was done to ensure that the analyte was stable in the biological matrix even after multiple freeze-thaw cycles.

1. Six quality control samples each at low and high concentrations stored below –50°C for at least 24 h were removed from the deep freezer and were allowed to thaw unassisted at room temperature (fT4 samples). These samples were frozen again below –50°C for at least 12 h.

2. Another set of six quality control samples at low and high concentration levels (fT3 samples) were removed from the deep freezer along with the fT4 samples and thawed unassisted. Both sets of samples were replaced back into the deep freezer.
3. At least after 12 h of freezing, fT4, fT3 and another set of six samples each at low and high concentration levels (fT2 samples) were removed from the deep freezer and thawed unassisted. All the samples were replaced back into the deep freezer.
4. At least after 12 h of freezing, fT4 samples were taken out from deep freezer, thawed unassisted to room temperature and analyzed with freshly prepared calibration curve (CC) solutions.

### 7.5.4. Bench Top (BT) Stability

Six quality control samples each at Quality Control sample at Low concentration (QCL) and Quality Control sample at High concentration (QCH) levels were stored at room temperature for 3 and 6 h. The above samples were analyzed along with freshly prepared calibration curve standards by using the method being validated.

### 7.5.5. Long-Term (LT) Stability

To assess the stability of the analyte in the biological matrix under the same conditions of storage as that of the study samples for the time period between the date of first sample collection and the date of last sample analysis, the following test was performed.

Six samples of each quality control samples at low and high concentrations were stored below -50°C in the deep freezer. The stability of the analyte was evaluated by comparing each of the back calculated concentrations of stability Quality Control sample (QCs) with the mean concentrations of the respective QCs analyzed in the first accepted precision and accuracy batch .

### 7.5.6. Auto sampler Stability

To evaluate the stability of the samples in the autosampler after processing for the anticipated run time, six sets of quality control samples each at low and high concentrations were placed in the auto sampler for 24 h and 48 h. The quality control samples were retained in the autosampler to prove auto sampler stability. After the lapse of the test time, the samples placed in the auto sampler were injected into the system along with freshly prepared calibration curve standards. The stability of the analyte was evaluated by comparing the back calculated concentration of stability samples from the freshly prepared calibration curve with their respective nominal concentrations.

To determine the auto sampler stability of the internal standard, the mean peak area obtained for the internal standard of the stability samples was compared with that of the mean of internal standard area of accepted non-zero calibration curve solution (CC's) and the percent change was calculated using the following expression:

% Change = 100 – {(Mean of internal standard peak area in the stability samples/ Mean of internal standard peak area of the accepted non – zero CCs) x 100)}

## 7.6. Recovery

Absolute recovery of a bio analytical method is the measured response obtained from a certain amount of analyte added to and extracted from the biological matrix, expressed as a percentage of the response obtained for the true concentration of the pure authentic standard which has not been subjected to the extraction procedure.

To determine recovery of this method, six replicates of aqueous quality control samples (un extracted) with concentrations close to spiked Quality Control sample at Low concentration (QCL), Quality Control sample at Middle concentration (QCM) and Quality Control sample at High concentration (QCH) concentration (extracted) were prepared. These un extracted samples were injected along with precision and accuracy batch.

% Recovery of analyte at each level was calculated using the following expression:

{[Individual analyte peak area of extracted QCs x Concentration of analyte added (un extracted sample)] / [Mean analyte peak area of aqueous QCs x Concentration of analyte added (extracted sample)]} x 100

The mean and standard deviation for the percent recovery obtained and there by the percent variation (%CV) was calculated at each concentration level. The overall percent recovery was calculated as the mean of recoveries obtained at the three quality control levels (QCL, QCM and QCH). The overall percent variation (% CV) was also calculated.

The percentage recovery for the internal standard was also calculated. The peak area response of the internal standard obtained for the extracted QCM sample (analyzed in the precision and accuracy batch) was compared with the mean area response of the internal standard obtained for the un extracted QCM samples.

### 7.7. Ruggedness

Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and column of similar type. Chromatographic parameters such as retention time, asymmetric factor, capacity factor and selectivity factor were evaluated.

### 7.8. Robustness

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of acetonitrile in the mobile phase, ± 0.5 units in the pH of the buffer, ± 0.5 ml volume of the triethylamine in aqueous phase and ± 0.1 ml of the flow rate.

**8.0. Method of analysis**

The processed standards and samples were analysed using optimised chromatographic conditions mentioned earlier and the chromatograms were recorded. The quantification of the chromatogram was performed using peak area ratios (response factor) of the drug to internal standard. The calibration curves were constructed routinely during the process of pre-study validation and in-study validation.

Analytical batch organization:

Samples were injected in the following order,

i) Aqueous standard

ii) Plasma blank

iii) Zero sample

iv) Calibration curve samples

v) Quality control samples

vi) Subject samples

**9.0. Development of *in vitro* dissolution methods**

The release characteristics of test and reference formulations of ondansetron hydrochloride and dextromethorphan hydrobromide were determined using USP XXIII dissolution apparatus (type II, paddle), at 50 and 75 rpm. The dissolution medium used were pH 1.2, 4.5, 5.5, 6.8 and 7.4 buffers maintained at 37±0.5˚C. Dissolution tests were performed on six tablets. Five ml of the samples were withdrawn at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h time intervals. Equal quantity of the dissolution medium was replaced to the dissolution jar after each sampling. The amount of the drug released was estimated by optimized and validated HPLC methods described in section 5.2. Percentage drug release and cumulative release at various time intervals were calculated and compared.

## 10.0. *In vivo* and *in vitro* data analysis

### 10.1. *In vivo* data analysis

The pharmacokinetic parameters $C_{max}$, $t_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$, $t_{1/2}$ and $k_{el}$ were determined using WinNonlin Standard edition version 5.1 for individual drug treatments from the observed plasma concentration-time data.

The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from time zero to the last concentration time point ($AUC_{(0-t)}$). The $AUC_{(0-t)}$ was determined by the trapezoidal method. $AUC_{(0-\infty)}$ was determined by the following equation:

$$AUC_{(0-\infty)} = AUC_{(0-t)} + C_{(t)} / k_{el}$$

$k_{el}$ was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline. The Wagner-Nelson method[52] was used to calculate the percentage of the dose absorbed,

$F_{(t)} = C_{(t)} + k_{el}\ AUC_{(0-t)}$ where $F_{(t)}$ is the amount absorbed.

The percent absorbed is determined by dividing the amount absorbed at any time by the plateau value, $k_e\ AUC_{(0-\infty)}$ and multiplying this ratio by 100

$$\% \text{ dose absorbed} = C_{(t)} + (k_{el}\ AUC_{(0-t)} / k_{el}\ AUC_{(0-\infty)}) \times 100$$

### 10.2. Statistical analysis of pharmacokinetic data

The statistical analysis using least square means (LSM) was carried out for each component of the test and reference product on the pharmacokinetic data obtained from six volunteers. The untransformed and log-transformed pharmacokinetic parameters ($C_{max}$, $AUC_{0-t}$, $AUC_{0-\infty}$) were analyzed by an Analysis of Variance (ANOVA) including the effects for treatments, sequence of dosing, subjects nested within sequences, period of treatment and drug formulations as factors in the statistical model.

The two one-sided 'T' tests for bioequivalence, 95% confidence intervals for the difference between treatments, least-square means were calculated for

ln-transformed $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ parameters. The confidence interval was expressed as a percentage relative to the LSM of the reference treatments.

### 10.3. *In vitro* dissolution data analysis

Percentage drug released or dissolved at various time intervals were calculated using the following formula,

Percentage release = {[Concentration (mg/ml) x bath volume (ml)] / Drug content (mg)} x 100

The dissolution profiles were determined by plotting the cumulative perc entage drug dissolved at various time points. The *in vitro* drug release profiles of the slow and fast modified release formulations (test formulations) were compared using the similarity factor, $f_2$, presented in the following equation,

$$f_2 = 50 \log \left[ 1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} X\ 100$$

where $R_t$ and $T_t$ are the cumulative percentage dissolved at each time point for the reference product and the test product, respectively.

The evaluation of the similarity is based on the following conditions;

- a minimum of three points (zero excluded),
- 12 individual values for every time points for each formulation,
- not more than one mean value of more than 85% dissolved for each formulation,
- the standard deviation of the mean of any formulation is less than

  10% from the second to last time points and
- in cases where more than 85 % of the drugs are dissolved within 15 min, dissolution profiles may be accepted without mathematical evaluation.

## 11.0. *In Vitro-In Vivo* Correlation (IVIVC)

### 11.1. IVIVC Model development

Linear regression analysis was used to examine the relationship between percentage of the drug dissolved and the percentage of drug absorbed. The percentage of the drug unabsorbed was calculated from the percentage absorbed. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. The dissolution rate constants were determined from % released versus the square root of time. Linear regression analysis was applied to the *in vitro-in vivo* correlation plots and the coefficient of correlation ($r^2$), slope and intercept values were calculated.

Level A correlation was estimated by a two-stage procedure, deconvolution followed by comparison of the percentage drug absorbed to the percentage drug dissolved.

### 11.2. IVIVC Model validation

The objective of any mathematical predictive tool is to successfully predict the outcome (*in vivo* profile) with a given model and test condition (*in vitro* profile). Integral to the model development exercise is model validation, which can be accomplished using data from the formulations used to build the model (internal validation) or using data obtained from a different (new) formulation (external validation). While internal validation serves the purpose of providing basis for the acceptability of the model, external validation is superior and affords greater "confidence" in the model.

#### 11.2.1. Internal validation

The predictability of the IVIVC was examined by using the mean *in vitro* dissolution data and mean *in vivo* pharmacokinetics of the selected modified release formulations. The mean *in vitro* dissolution rate constants was correlated with the mean absorption rate constants for the modified release formulations.

These two data points, along with the zero-zero intercept were used to calculate the expected absorption rate constants.

The prediction of plasma concentration was accomplished using the following curve fitting equation:

$y = \text{Const. X (Dose) X } k_a / k_a - k_{el} (e^{-kelt} - e^{-kat})$

where, y = predicted plasma concentration (ng/ml); Const. = the constant representing F / Vd (where F is the fraction absorbed, and Vd is the volume of distribution); $k_a$= absorption rate constant; $k_{el}$= overall elimination rate constant.

To further assess the predictability and the validity of the correlations, the observed and IVIVC model-predicted $C_{max}$ and AUC values for formulation are determined. The percent prediction errors for $C_{max}$ and AUC were calculated as follows:

$$\%\ PE_{Cmax} = \left( \frac{C_{max}\ (obs) - C_{max}\ (pred)}{C_{max}\ (obs)} \right) X\ 100$$

$$\%\ PE_{AUC} = \left( \frac{AUC\ (obs) - AUC\ (pred)}{AUC\ (obs)} \right) X\ 100$$

where $C_{max}$ (obs) and $C_{max}$ (pred) are the observed and IVIVC model-predicted maximum plasma concentrations, respectively; and AUC (obs) and AUC (pred) are the observed and IVIVC model-predicted AUC for the plasma concentration profiles, respectively.

The criteria set in the FDA guidance on IVIVC for level A are as follows: For $C_{max}$ and AUC, the mean absolute percent prediction error(% PE) should not exceed 10% and the prediction error for individual formulations should not exceed 15%.

### 11.2.2. External Validation

For establishing external predictability, the exposure parameters for a new formulation are predicted using its *in vitro* dissolution profile, the IVIVC model and the predicted parameters are compared to the observed parameters. The prediction errors are computed as for the internal validation. For $C_{max}$ and AUC, the prediction errors for the external validation formulation should not exceed 10%. A prediction error of 10% to 20% indicates inconclusive predictability and illustrates the need for further study using additional data sets. For drugs with narrow therapeutic index, external validation is required despite acceptable internal validation, whereas internal validation is usually sufficient with non-narrow therapeutic index drugs.

**Criteria**

- % Prediction Error (PE) of 10% or less for $C_{max}$ and AUC establishes the external predictability of an IVIVC.
- % PE between 10 - 20% indicates inconclusive predictability and the need for further study using additional data sets. Results of estimation of PE from all such data sets should be evaluated for consistency of predictability.
- % PE greater than 20% generally indicates inadequate predictability, unless otherwise justified.

**Figure 1 Photograph of Ondansetron hydrochloride and Dextromethorphan Hydrobromide modified release tablets**

**Table 1 Formulation prepared by wet granulation method ($F_1$-$F_{17}$) for Ondansetron hydrochloride (OND)**

| F[a] | OND | HPMC | Carbopol | Avicel | Magnesium Stearate | Aerosil | PVP-k-30 | Total (mg/tab) |
|---|---|---|---|---|---|---|---|---|
| $F_1$ | 8 | 5 | ------ | 75.5 | 1 | 0.5 | 10 | 100 |
| $F_2$ | 8 | 10 | ------ | 70 | 1 | 0.5 | 10 | 100 |
| $F_3$ | 8 | 15 | ------ | 65.5 | 1 | 0.5 | 10 | 100 |
| $F_4$ | 8 | 20 | ------ | 60.5 | 1 | 0.5 | 10 | 100 |
| $F_5$ | 8 | 25 | ------ | 55.5 | 1 | 0.5 | 10 | 100 |
| $F_6$ | 8 | 30 | ------ | 50.5 | 1 | 0.5 | 10 | 100 |
| $F_7$ | 8 | ------ | 5 | 75.5 | 1 | 0.5 | 10 | 100 |
| $F_8$ | 8 | ------ | 10 | 70 | 1 | 0.5 | 10 | 100 |
| $F_9$ | 8 | ------ | 15 | 65.5 | 1 | 0.5 | 10 | 100 |
| $F_{10}$ | 8 | ------ | 20 | 60.5 | 1 | 0.5 | 10 | 100 |
| $F_{11}$ | 8 | ------ | 25 | 55.5 | 1 | 0.5 | 10 | 100 |
| $F_{12}$ | 8 | ------ | 30 | 50.5 | 1 | 0.5 | 10 | 100 |
| $F_{13}$ | 8 | 2.5 | 2.5 | 75.5 | 1 | 0.5 | 10 | 100 |
| $F_{14}$ | 8 | 5 | 5 | 70 | 1 | 0.5 | 10 | 100 |
| $F_{15}$ | 8 | 7.5 | 7.5 | 65.5 | 1 | 0.5 | 10 | 100 |
| $F_{16}$ | 8 | 10 | 10 | 60.5 | 1 | 0.5 | 10 | 100 |
| $F_{17}$ | 8 | 12.5 | 12.5 | 55.5 | 1 | 0.5 | 10 | 100 |

[a] Code of formulations

**Table 2 Formulation prepared by wet granulation method ($F_1$-$F_{18}$) for Dextromethorphan hydrobromide (DEX)**

| F[a] | DEX | HPMC | Carbopol | Avicel | Magnesium Stearate | Aerosil | PVP-k-30 | Total (mg/tab) |
|---|---|---|---|---|---|---|---|---|
| $F_1$ | 60 | 15 | ------ | 102 | 2 | 1 | 20 | 200 |
| $F_2$ | 60 | 30 | ------ | 87 | 2 | 1 | 20 | 200 |
| $F_3$ | 60 | 45 | ------ | 72 | 2 | 1 | 20 | 200 |
| $F_4$ | 60 | 60 | ------ | 57 | 2 | 1 | 20 | 200 |
| $F_5$ | 60 | 90 | ------ | 27 | 2 | 1 | 20 | 200 |
| $F_6$ | 60 | 117 | ------ | ------ | 2 | 1 | 20 | 200 |
| $F_7$ | 60 | ------ | 15 | 102 | 2 | 1 | 20 | 200 |
| $F_8$ | 60 | ------ | 30 | 87 | 2 | 1 | 20 | 200 |
| $F_9$ | 60 | ------ | 45 | 72 | 2 | 1 | 20 | 200 |
| $F_{10}$ | 60 | ------ | 60 | 57 | 2 | 1 | 20 | 200 |
| $F_{11}$ | 60 | ------ | 90 | 27 | 2 | 1 | 20 | 200 |
| $F_{12}$ | 60 | ------ | 117 | ------ | 2 | 1 | 20 | 200 |
| $F_{13}$ | 60 | 7.5 | 7.5 | 102 | 2 | 1 | 20 | 200 |
| $F_{14}$ | 60 | 15 | 15 | 87 | 2 | 1 | 20 | 200 |
| $F_{15}$ | 60 | 22.5 | 22.5 | 72 | 2 | 1 | 20 | 200 |
| $F_{16}$ | 60 | 30 | 30 | 57 | 2 | 1 | 20 | 200 |
| $F_{17}$ | 60 | 45 | 45 | 27 | 2 | 1 | 20 | 200 |
| $F_{18}$ | 60 | 58.5 | 58.5 | ------ | 2 | 1 | 20 | 200 |

[a] Code of formulations

**Table 3 Products of Bioavailability Evaluation**

| Name of the Drug | Reference Product A (Immediate release formulations) | Test B (Slow modified release formulations) | Test C (Fast modified release formulations) |
|---|---|---|---|
| Ondansetron hydro chloride | EMSETRON (Sun pharmaceutical Ltd, Mumbai, India) tablets containing 8 mg of Ondansetron hydrochloride | In-house tablets (Manufactured by J.S.S.College of Pharmacy, Ootacamund, India) containing 8 mg of Ondansetron hydro chloride | In-house tablets (Manufactured by J.S.S.College of Pharmacy, Ootacamund, India) containing 8 mg of Ondansetron hydro chloride |
| Dextromethorphan hydro bromide | ROMILAR (Roche Pharma, South Africa) tablets containing 15 mg of Dextromethorphan hydrobromide | In-house tablets (Manufactured by J.S.S.College of Pharmacy, Ootacamund, India) containing 60 mg of Dextromethorphan hydro bromide | In-house tablets (Manufactured by J.S.S.College of Pharmacy, Ootacamund, India) containing 60 mg of Dextromethorphan hydro bromide |

**Figure 2 UV spectrum of Ondansetron hydrochloride**

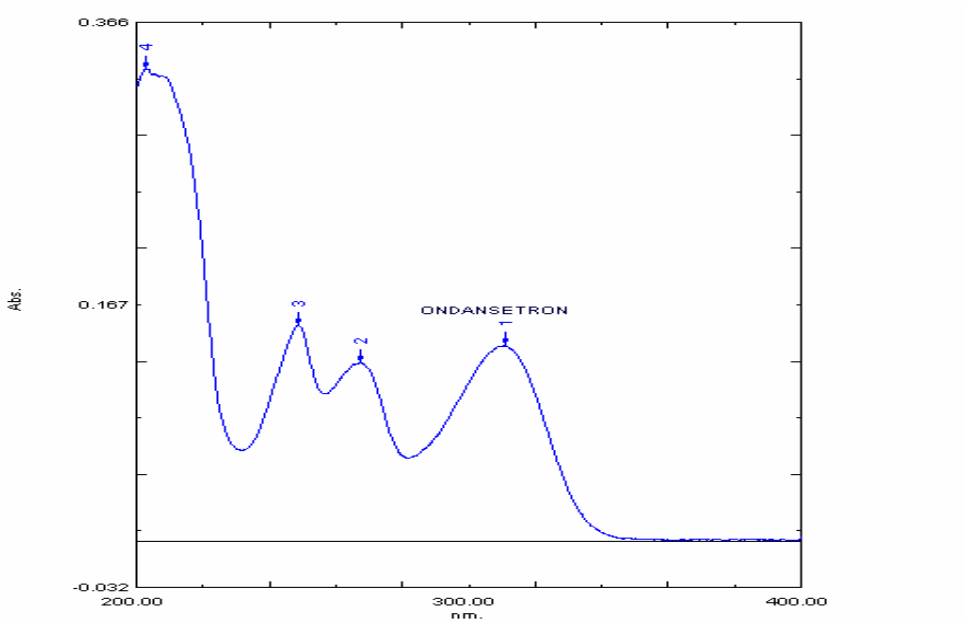



**Figure 3 UV spectrum of Dextromethorphan hydrobromide**

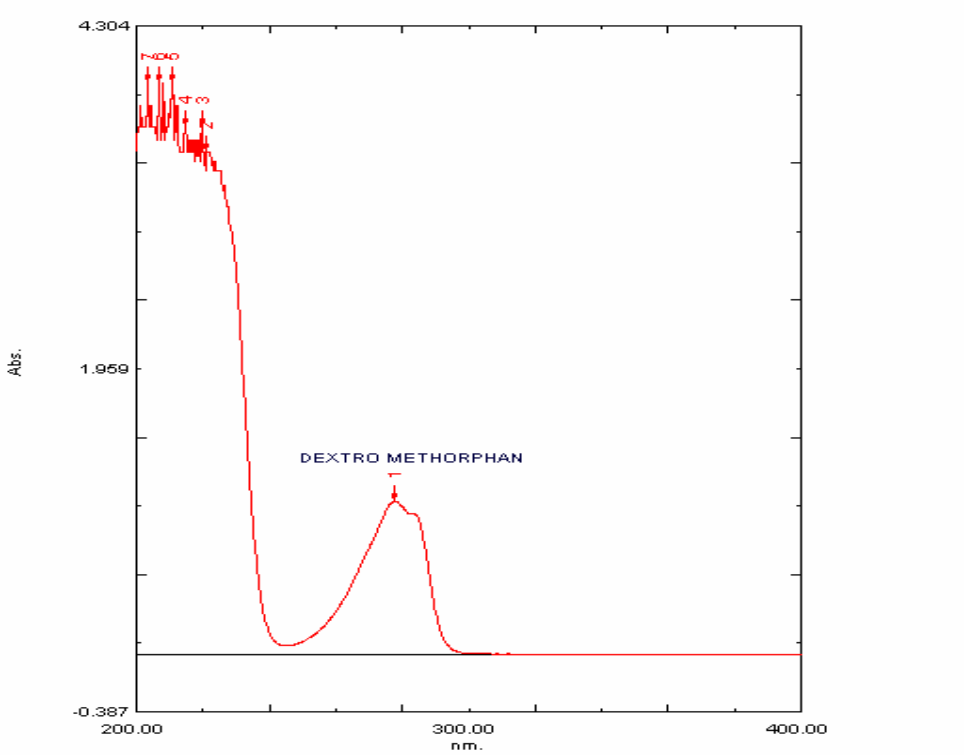


**Table 4 Mobile phase composition**

| S. No | Drug Name | Ratio of the Mobile phase |
|---|---|---|
| 1 | Ondansetron hydro chloride | Acetonitrile-25 mM phosphate buffer (pH 7.0), 60:40 (v/v) |
| 2 | Dextromethorphan hydrobromide | Acetonitrile - 0.1% trifluoroacetic acid, (40:60, v/v) |

# 7. RESULTS

This chapter describes the experimental results obtained in the present investigation in the form of Tables and Figures along with a detailed analysis on results of preformulation study, tablet manufacture, bioavailability study design, data handling, optimization and validation of the bio analytical methods for the estimation of ondansetron hydrochloride and dextromethorphan hydrobromide in human plasma samples, amount of the selected drugs present in plasma samples, *in vitro* dissolution method, determination of pharmacokinetic parameters, statistical evaluation, *in vivo* and *in vitro* data analysis, *In Vitro - In Vivo* Correlation (IVIVC) model development and validation of level A IVIVC.

## 1.0. Evaluation of Granulations

Granulation is the key process in the production of many dosage forms. The sustained release tablets were prepared by wet granulation technique. Physical properties of granules such as specific surface area, shape, hardness, surface characteristics and size can significantly affect the rate of dissolution of drugs contained in a heterogeneous formulation. The granules of two different formulations were evaluated for angle of repose, loose bulk density (LBD), tapped bulk density (TBD) and Carr`s index as shown in Table 5 & 6 of ondansetron hydrochloride and dextromethorphan hydrobromide respectively.

### 1.1. DSC studies

No significant changes in terms of peak shifting, appearance or disappearance of peaks were noted with the two drugs, polymers and mixtures. This confirmed the absence of chemical interaction between the selected drugs, excipients and polymers. Absence of incompatibility between the selected drugs and polymers was also confirmed by the DSC pattern matching approach. DSC spectra are given in Figure 4a, 4b & 5a, 5b of ondansetron hydrochloride and dextromethorphan hydrobromide respectively.

### 1.2 Compatibility studies

The initial IR spectra of the drug and the polymer are satisfactory with their characteristic absorption bands. Similarly, the physical mixtures also indicate the presence of characteristic peaks of the drug and the polymer. It is clear that the drug and the excipients are free from any significant chemical interactions. The IR spectra are given in Figure 6a, 6b & 7a, 7b of ondansetron hydrochloride and dextromethorphan hydrobromide respectively.

## 2.0. Tablet Manufacture

### 2.1. Development of Ondansetron hydrochloride and Dextromethorphan Hydrobromide SR tablets

The physical properties of different batches of developed tablets are given in Table 7 & 8 of ondansetron hydrochloride and dextromethorphan hydrobromide respectively. All the batches showed uniform thickness. The average percentage deviation of 20 tablets of each formula was less than ± 5% and hence all formulations passed the test for uniformity of weight as per official requirements (Pharmacopoeia of India 1996). Good uniformity content was found among three different batches of tablets. Another measure of tablets strength is

friability. In the present study, the percentage friability for all the formulations was below 1%, indicating that the friability is within the prescribed limits**.** All the tablets formulations showed acceptable pharmaco technical properties and complied with the specifications for weight variation, drug content, hardness and friability.

**2.2. *In vitro* release**

A suitable *in vitro* dissolution method serves as a valuable quality control tool to assess batch to batch release performance and to assure the physiological availability of the drug. The *in vitro* dissolution test is also used to guide formulation development and to monitor manufacturing process. As a regulatory test, it is used to approve minor changes in formulation, changes in the site of manufacturing and also to assess the scale-up of the bio-batch to the production batch.

All the batches have shown that as the polymer concentration increases, the drug release rate decreases for ondansetron hydrochloride and dextromethorphan Hydrobromide. (Figure 8 - 13).

The *in vitro* drug release characteristics of the developed sustained release (SR) and the marketed immediate release (IR) tablets were studied. Dissolution data for all the experiments were highly reproducible and hence only the average values were plotted. The dissolution of the marketed IR tablets indicated that more than 80% of the drug is released within 1h, which complies with the pharmacopoeial specifications. In all the batches, we observed that as the polymer concentration increases, the drug release rate decreases.

To know the mechanism of drug release from these formulations, the data were treated according to zero-order (cumulative amount of drug released versus time), first-order (log cumulative percentage of drug released versus time), Higuchi (Cumulative percentage of drug released versus square root of time) and Peppas (log cumulative percentage of drug released versus log time) equations which are clearly revealed in Figure 14-21 for ondansetron hydrochloride and

dextromethorphan hydrobromide. The optimized formulations $F_1$ & $F_4$ for ondansetron hydrochloride and dextromethorphan hydrobromide were subjected to stability studies at different temperature and humidity conditions as per ICH guidelines. *In vivo* studies were carried out for the optimized formulation in six healthy human volunteers and the pharmacokinetic studies were carried out for the optimized formulation and compared with the internationally marketed formulation.

**2.3. Kinetics and Mechanism of Drug Release**

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted in various kinetics models: zero order (equation 1) as cumulative amount of drug released versus time, first order (equation 2) as log cumulative percentage of drug remaining versus time and Higuchi`s model (equation 3) as cumulative percentage of drug released versus square root of time.

$$Q_t = k_0 t \qquad (1)$$

where, Q is the amount of drug release in time t and $K_0$ is the zero - order rate constant and t is the time in hours.

$$\ln Q_t = \ln Q_0 - k_1 . t \qquad (2)$$

where $Q_0$ is the initial concentration of drug and $k_1$ is the first order rate constant.

$$Q_t = K_2 t^{1/2} \qquad (3)$$

where $K_2$ is the rate constant of Higuchi equation. Hence, drug release rate is proportional to the reciprocal of the square root of time.

The *in vitro* drug release profiles were plotted according to zero – order, first- order, Higuchi and Peppas equations to understand the mechanism of drug release and to compare the differences in the release profile of optimized batches of ondansetron hydrochloride and dextromethorphan hydrobromide tablets (Figure 14-21) respectively.

## 2.4. Stability Studies

No significant change was observed for the formulated sustained release tablets of ondansetron hydrochloride and dextromethorphan hydrobromide with respect to its physicochemical parameters and *in vitro* drug release as evident by Table 9 & 10. The developed formulations for ondansetron hydrochloride and dextromethorphan hydrobromide are, therefore, stable at various temperature and humidity conditions for a period of 3 months.

### 2.5. Bioavailability study design and data handling

A single dose, randomized, complete, three treatments cross over study was conducted in healthy human subjects for the selected drug formulations. Six volunteers aged between 20-30 years were selected. Seven days prior to the commencement of the study, volunteers were subjected to preliminary screening, standard clinical and biochemical investigations.

After overnight fasting, the volunteers were given code numbers and allocated to the treatment in accordance with the randomized code. The order of treatment administration was randomized in three sequences (ABC, BCA, and CAB) in blocks of three. In each dosing session, volunteers received Reference Product A (Immediate release formulations), Test B (Slow modified release formulations) and Test C (Fast modified release). A wash out period of seven days was allowed between dose administrations. Blood samples (4 ml) were collected at 0 (before drug administration), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h post dosing. The samples were centrifuged and plasma was separated. There were no serious adverse effects observed during the entire study.

### 2.6. Estimation of the selected drugs in human plasma

#### 2.6.1. Optimization of chromatographic conditions

Optimization of the chromatographic conditions are intended to take into account the various goals of method development and to weigh each goal (resolution, runtime, sensitivity, peak symmetry, etc) accurately, according to the requirement of HPLC methods being used for the estimation of drugs in biological fluids. Reversed phase HPLC method was chosen for ondansetron hydrochloride and dextromethorphan hydrobromide.

The standard solutions of ondansetron hydrochloride and dextromethorphan hydrobromide were scanned from 200–400 nm and the UV spectra obtained were

recorded. From the UV spectra, the detection wavelength selected was 305 nm and 280 nm for ondansetron hydrochloride and dextromethorphan hydrobromide, respectively. The wavelength selected gave good peak response.

Acetonitrile or methanol was selected as organic phase in the mobile phase to elute the drugs from the stationary phase because of its favorable UV transmittance, low viscosity and better solubility for the selected drugs. The pH of the initial mobile phase selected was 2.0 because a low pH protonates column silanols (free hydroxyl group in reverse phase column) and reduces their chromatographic activity, it forms hydrogen bonds with the polar groups leading to peak tailing. Further, a low pH (less than 3) is usually quite different from the pka values of the weakly acidic drugs under study. At low pH, therefore, the retention of drugs will not be affected by slow changes in pH and the RP-HPLC methods will be more rugged.

The standard solutions were analyzed using the initial chromatographic conditions. To improve the resolution or symmetry of the peaks or to study the effect of the other chromatographic conditions, the chromatographic variables like pH of the mobile phase, the nature of stationary phase, the composition of the mobile phase, flow rate and selection of internal standard were optimized.

### 2.6.2. Validation of HPLC methods

Estimation of the drugs selected in plasma samples from the volunteers was carried out using optimized chromatographic conditions. The validation parameters such as accuracy, precision (repeatability and reproducibility), linearity and range, sensitivity (limit of detection and limit of quantitation), robustness/ruggedness, stability, selectivity/specificity and system suitability were evaluated.

### 2.6.2.1. Ondansetron hydrochloride

#### 2.6.2.1.1. Specificity

HPLC-UV analysis of the blank human plasma samples showed the separation of ondansetron hydrochloride and etoricoxib, no interference with either of these were observed. Hence the specificity of the method was established by comparison with human plasma (control). Representative chromatograms of extracted blank plasma, blank plasma fortified with internal standard (IS) are shown in Figure 22a - 22f indicating no interference in the blank plasma and in drug-free human plasma at the retention time of 6.8 for the drug ondansetron hydrochloride and at the retention time of 13.1 for the IS.

#### 2.6.2.1.2. Sensitivity

The limit of reliable quantitation was set at the concentration of the LOQ QC, 0.50 ng/ml for ondansetron and lowest non-zero standard.

#### 2.6.2.1.3. Linearity

A regression equation with a weighing factor of 1/concentration$^2$ was judged to produce the best fit for the concentration/detector response relationship for ondansetron in human plasma. The linearity range for ondansetron was found to be 0.5, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0 and 50.0 ng/ml. The results are given in Table 11 and is shown in Figure 23 with correlation coefficient ($r^2$) was greater than 0.99.

#### 2.6.2.1.4. Precision and Accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentration range of LOQ, low, middle and high quality control sample of ondansetron during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LOQ, low, middle and high quality control samples to their respective nominal values, expressed as percent. The results are given in Table 12a – 12c.

#### 2.6.2.1.5. Stabilities

The stability studies of plasma samples spiked with selected drugs were subjected to three freeze-thaw cycles, short term stability at room temperature for 3 h and long term stability at – 70$^{o}$C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 h and freeze condition for four weeks. The mean concentrations of the stability samples were compared to the theoretical concentrations. The results indicate that selected drugs in plasma samples can be stored for a month without degradation in frozen state. The results of short term storage at room temperature stability and freeze-thaw cycles indicate no degradation of selected drugs in plasma as well as in sample solution and hence plasma samples could be handled without special precautions. The results are given in Table 13a -13f.

#### 2.6.2.1.6. Recovery

Analyte recovery from a sample matrix (extraction efficiency) is a comparison of the analytical response from an amount of analyte added to that determined from the sample matrix. The detailed results are presented in Table 14a-14b. The results indicate that the recovery of ondansetron was consistent at all levels.

#### 2.6.2.1.7 Ruggedness and robustness

The ruggedness and robustness of the methods were studied by changing the experimental conditions. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, instruments, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate).

### 2.6.2.2 Dextromethorphan hydrobromide

#### 2.6.2.2.1 Specificity

HPLC-UV analysis of the blank human plasma samples showed the separation of dextromethorphan hydrobromide and losartan potassium and no interference with either of these were observed. Hence the specificity of the method was established by comparison with human plasma (control). Representative chromatograms of extracted blank plasma, blank plasma fortified with internal standard (IS) are shown in Figure 24a – 24d indicating no interference in the blank plasma and in drug-free human plasma at the retention time of 6.2 for the drug dextromethorphan hydrobromide and at the retention time of 9.7 for the IS.

#### 2.6.2.2.2 Sensitivity

The limit of reliable quantitation was set at the concentration of the LOQ QC, 110.0 ng/ml for dextromethorphan and lowest non-zero standard.

#### 2.6.2.2.3 Linearity

A regression equation with a weighing factor of $1/concentration^2$ was judged to produce the best fit for the concentration/detector response relationship for dextromethorphan hydrobromide in human plasma. The linearity range for dextromethorphan hydrobromide was found to be 110.0, 140.0, 230.0, 590.0, 1000.0, 1400.0, 2100.0 and 3000.0 ng/ml. The results are given in Table 15 and is shown in Figure 25 with correlation coefficient ($r^2$) was greater than 0.99.

#### 2.6.2.2.4 Precision and Accuracy

The precision of the assay was measured by the percent coefficient of variation over the concentration range of LOQ, low, middle and high quality control sample of dextromethorphan hydrobromide during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LOQ, low, middle and high quality control samples to their respective nominal values, expressed as percent. The results are given in Table 16a – 16c.

#### 2.6.2.2.5 Stabilities

The stability studies of plasma samples spiked with selected drugs were subjected to three freeze-thaw cycles, short term stability at room temperature for 3 h and long term stability at – 70$^{o}$C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 h and freeze condition for four weeks. The mean concentrations of the stability samples were compared to the theoretical concentrations. The results indicate that selected drugs in plasma samples can be stored for a month without degradation in frozen state. The results of short term storage at room temperature stability and freeze-thaw cycles indicate no degradation of selected drugs in plasma as well as in sample solution and hence plasma samples could be handled without special precautions. The results are given in Table 17a – 17f.

#### 2.6.2.2.6 Recovery

Analyte recovery from a sample matrix (extraction efficiency) is a comparison of the analytical response from an amount of analyte added to that determined from the sample matrix. The detailed results are presented in Table 18a-18b. The results indicate that the recovery of dextromethorphan was consistent at all levels.

#### 2.6.2.2.7 Ruggedness and robustness

The ruggedness and robustness of the methods were studied by changing the experimental conditions. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, instruments, source of reagents and column of similar type) and optimized conditions (pH, mobile phase ratio and flow rate).

In conclusion, the developed methods for the estimation of ondansetron hydrochloride and dextromethorphan hydrobromide in plasma are accurate, precise, selective, linear and hence they are useful for bioavailability studies.

**2.7. Estimation of selected drugs in plasma samples**

The calibration curve samples (CC samples), quality control samples (QC samples) and plasma sample solutions were injected with the optimized & validated chromatographic conditions and the chromatograms were recorded. The quantification of the chromatogram was performed using peak area ratios (response factor) of the drug to internal standard. The calibration curves were constructed routinely for spiked plasma containing the drug candidates and internal standard during the process of pre-study validation and in-study validation. The mobile phase used for the assay provided a well defined separation between the drug, internal standard and endogenous components. The zero h (pre dose) samples of all subjects showed no interference at retention time of both selected drugs and internal standards. The individual and mean concentration of the drugs present in the plasma samples were calculated and are presented in the Tables 19-26.

**2.8. *In vivo* data analysis**

Pharmacokinetic parameters such as peak plasma concentration ($C_{max}$), time to peak concentration ($t_{max}$), area under the plasma concentration-time curve ($AUC_{0-t}$ & $AUC_{0-\infty}$), elimination rate constant ($k_{el}$) and elimination half-life ($t_{1/2}$) were calculated separately and the blood level data of selected formulations were compared and are presented in the Table 27.

Mean plasma concentration-time profile of ondansetron hydrochloride and dextromethorphan hydrobromide were given in Figures 26 & 27.

The mean pharmacokinetic profile and parameters for the slow modified release (MR) test formulations and the reference formulations were relatively different. However, the profile for the fast MR test formulations displayed a faster rate of absorption compared with the slow MR test formulations. There was thus a

decrease in the absorption for the MR formulations when compared to the immediate release (IR) reference formulations.

The ln-transformed values of $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ along with the factors included in this statistical analysis were periods, sequences, treatments and subjects. The factor subject was random and others were fixed. A difference between the treatments was calculated including the 95% confidence interval of that difference. The design statement indicates that the subjects were nested within the sequences as discussed below:

### 2.8.1 Ondansetron hydrochloride

The statistical parameters for ln-transformed values of $C_{max}$ like sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of ondansetron hydrochloride between subject effects are given in Tables 28 - 39. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast modifies release (MR) test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $C_{max}$ values for individual subjects and mean percentage ratio are presented in the tables. The 95% confidence interval for slow, fast test formulations and reference formulation ranges from 0.01254 to 0.18080 and from -0.00218 to 0.21218, respectively, while the mean differences for slow, fast test formulations and reference formulation were 0.09667 and 0.10500, respectively. Back transformed to regular units, this means that the mean $C_{max}$-0.09667 =1.10149 and 0.10500=11071, while the 95% confidence interval for slow, fast versus reference formulations ranges from 0.01254 =1.01261 to 0.18080 =1.19817 and from -0.00218=1.00218 to 0.21218= 1.23637, respectively. The mean percentage ratio between slow, fast versus reference formulations were 110.14 and 110.71, respectively. The percentage confidence interval for slow, fast versus reference formulations ranges from 91.93 to 108.77 and from 89.83 to 110.82 respectively.

The statistical parameters for ln-transformed values of $AUC_{0-t}$ like the sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of ondansetron hydrochloride between subjects effects are given in the tables. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast MR test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $AUC_{0-t}$ values for individual subjects and mean percentage ratio are presented in the tables. The 95% confidence interval for slow, fast test formulations and reference formulation ranges from 0.72527 to 1.14139 and from 0.78339 to 0.99661, respectively, while the mean differences for slow, fast test formulations and reference formulation were 0.9333 and 0.8900, respectively. Back transformed to regular units, this means that the mean $AUC_{0-t}$ -0.9333 =2.5429 and 0.8900=2.4351, while the 95% confidence interval for slow, fast versus reference formulations ranges from 0.72527=2.0652 to 1.14139 =3.1311 and from -0.78339=2.1888 to 0.99661= 2.7090, respectively. The mean percentage ratio between slow, fast versus reference formulations were 254.30 and 243.51, respectively. The percentage confidence interval for slow, fast versus reference formulations ranges from 81.21 to 123.12 and from 89.88 to 111.25 respectively.

The statistical parameters for ln-transformed values of $AUC_{0-\infty}$ like the sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of ondansetron hydrochloride between subject effects are given in the tables. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast MR test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $AUC_{0-\infty}$ values for individual subjects and mean percentage ratio are presented in the tables. The 95% confidence interval for slow, fast test formulations and reference formulation ranges from 0.73139 to 1.12195 and from 0.79631 to 0.99661, respectively, while the

mean differences for slow, fast test formulations and reference formulation were 0.92667 and 0.89333, respectively. Back transformed to regular units, this means that the mean $AUC_{0-\infty}$ -0.92667 =2.5260 and 0.89333=2.4432, while the 95% confidence interval for slow, fast versus reference formulations ranges from 0.73139 =2.0779 to 1.1219 =3.0708 and from 0.79631=2.2173 to 0.99035= 2.6921, respectively. The mean percentage ratio between slow, fast versus reference formulations were 252.60 and 244.32, respectively. The percentage confidence interval for slow, fast versus reference formulations ranges from 82.26 to 121.56 and from 90.75 to 110.18 respectively.

### 2.8.2 Dextromethorphan hydrobromide

The statistical parameters for ln-transformed values of $C_{max}$ like the sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of dextromethorphan hydrobromide between subject effects are given in Tables 40 - 51. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast modified release (MR) test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $C_{max}$ values for individual subjects and mean percentage ratio are presented in the tables. The 95% confidence interval for slow, fast test formulations and reference formulation ranges from 0.46175 to 0.59158 and from 0.51723 to 0.63277, respectively, while the mean differences for slow, fast test formulations and reference formulation were 0.52667 and 0.57500, respectively. Back transformed to regular units, this means that the mean $C_{max}$ . 0.5266 = 1.69328 and .57500=1.7771, while the 95% confidence interval for slow, fast versus reference formulations ranges from 0.46175 =1.58684 to 0.59158 =1.8068 and from -0.51723=1.67737 to 0.63277= 1.88281, respectively. The mean percentage ratio between slow, fast versus reference formulations were 169.32 and 177.71,

respectively. The percentage confidence interval for slow, fast versus reference formulations ranges from 93.71 to 106.70 and from 94.38 to 105.56 respectively.

The statistical parameters for ln-transformed values of $AUC_{0-t}$ that is the sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of dextromethorphan hydrobromide between subject effects are given in the tables. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast MR test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $AUC_{0-t}$ values for individual subjects and mean percentage ratio are presented in the tables. The 95% confidence interval for slow, fast test formulations and reference formulation ranges from 1.5558 to 1.8741 and from 1.46819 to 1.66181, respectively, while the mean differences for slow, fast test formulations and reference formulation were 1.5650 and 1.7150, respectively. Back transformed to regular units, this means that the mean $AUC_{0-t}$ 1.5650 =4.7826 and 1.7150=5.5566, while the 95% confidence interval for slow, fast versus reference formulations ranges from 1.46819=4.3413 to 1.6618 =5.2687 and from 1.5558=4.7392 to 1.8741= 6.5150, respectively. The mean percentage ratio between slow, fast versus reference formulations were 478.26 and 555.66, respectively. The percentage confidence interval for slow, fast versus reference formulation ranges from 90.77 to 110.16 and from 85.28 to 117.24 respectively.

The statistical parameters for ln-transformed values of $AUC_{0-\infty}$ like the sum of square, degree of freedom, mean square, F, significance values for slow, fast release test formulations and reference formulation of dextromethorphan hydrobromide between subject effects are given in the tables. From these values, it is seen that the period, sequence and treatment effects are non-significant when slow, fast MR test formulation was compared with reference formulation. The 95% confidence interval of the difference between the two ln-tranformed $AUC_{0-\infty}$ values for individual subjects and mean percentage ratio are presented in the tables. The

95% confidence interval for slow, fast test formulations and reference formulation ranges from 1.74071 to 1.98655 and from 1.86336 to 2.10635, respectively, while the mean differences for slow, fast test formulations and reference formulation were 1.8633 and 1.9700, respectively. Back transformed to regular units, this means that the mean $AUC_{0-\infty}$ 1.8633 = 6.4451 and 1.9700=7.1706, while the 95% confidence interval for slow, fast versus reference formulations ranges from 1.74071 =5.6979 to 1.98655 =7.2903 and from 1.8336=6.2566 to 2.1063= 8.2181, respectively. The mean percentage ratio between slow, fast versus reference formulations were 644.51 and 717.06, respectively. The percentage confidence interval for slow, fast versus reference formulation ranges from 88.40 to 113.11 and from 87.25 to 114.60 respectively.

## 2.9. *In vitro-in vivo* correlations

This section describes *in vitro* and *in vivo* data analysis, *in vitro-in vivo* model 'A' correlation development and validation for selected formulations containing ondansetron hydrochloride and dextromethorphan hydrobromide.

### 2.9.1. Ondansetron hydrochloride

The *in vitro* release characteristics of the slow test modified release (MR) and fast test MR formulations of ondansetron hydrochloride were determined. Cumulative percentage drug release at various time intervals were calculated and are presented in Tables 52 & 53 and in Figures 28 & 29. The similarity factor ($f_2$) was calculated and is presented in Table 54.

When dissolution tests were performed at pH 1.2 buffer, pH 6.8 buffer and water at 50 and 75 rpm, the release of the ondansetron hydrochloride was found to be almost indistinguishable between the slow and fast formulations. The $f_2$ value for 1.2 buffer, pH 6.8 buffer and water at 50 rpm was 38.22,51.18 and 52.23, respectively, whereas at 75 rpm, the $f_2$ value was 51.06, 60.97 and 70.52, respectively. The higher $f_2$ values (more than 50) confirms that these dissolution mediums are indistinguishable and ensures sameness or equivalence between the two dissolution profiles and hence not considered for the present study.

The best discrimination was achieved at pH 4.5 buffer, pH 7.4 buffer at 50 rpm as well as 75 rpm. The $f_2$ value for pH 4.5 buffer and pH 7.4 buffer at 50 rpm was 34.23 and 38.93, respectively whereas at 75 rpm, the $f_2$ value was 36.19 and 42.54, respectively. The associated $f_2$ metric, an $f_2$ value below 50 suggests that the two dissolution profiles are dissimilar and reveals pH 4.5 buffer and pH 7.4 buffer at 50 and 75 rpm were more discriminating dissolution mediums and hence selected for IVIVC model development.

Level A correlation was developed by a two-stage procedure, deconvolution followed by comparison of the percent dissolved versus the percent absorbed data for both the slow and fast formulations. The *in vitro- in vivo* correlation plot was constructed using percentage of drug dissolved at pH 4.5 buffer dissolution media at both 50 and 75 rpm versus the percentage of drug absorbed. The slope of the best-fit line was examined using linear regression analysis and coefficient of correlation ($r^2$). The slope and intercept values were calculated and are presented in Tables 55 & 56 and in Figures 30 & 31. The correlation coefficient ($r^2$) for pH 4.5 buffer at 50 rpm and 75 rpm was 0.9032 and 0.7985, respectively. A good linear regression relationship was thus observed when the dissolution studies were carried out in pH 4.5 buffer at 50 rpm and hence this was selected for further analysis.

The dissolution rate constants were determined from percentage drug released versus the square root of time. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. Linear regression analysis was applied to the *in vitro- in vivo* correlation plots and coefficient of correlation ($r^2$), slope and intercept values were calculated and are presented in Figures 32-34. The correlation coefficient ($r^2$) for pH 4.5 buffer at 50 was 0.9988. A good linear regression relationship was thus observed using pH 4.5 buffer as dissolution medium at 50 rpm and hence this was selected as the dissolution media of choice.

#### 2.9.1.1. Internal Validation

The predictability of the IVIVC was examined by using the mean *in vitro* dissolution data and mean *in vivo* pharmacokinetics of the selected modified release formulations. The mean *in vitro* dissolution rate constants were correlated to the mean absorption rate constants for the modified release formulations. These two data points, along with the zero-zero intercept were used to calculate the expected absorption rate constants.

The prediction of plasma concentration was calculated. From this, percentage prediction errors for $C_{max}$ and AUC were calculated and are presented in Tables 57 & 58 and in Figures 35 & 36. The $C_{max}$ prediction errors for both the slow and fast formulations were found to be -7.91 and -8.70, respectively. These values were very close to the observed mean values. The AUC prediction error was 8.44 and 9.27 % for slow and fast formulations, respectively.

The $C_{max}$ and AUC prediction error was within the specified limit and hence, the IVIVC is considered as validated.

### 2.9.2. Dextromethorphan Hydrobromide

The *in vitro* release characteristics of the slow test modified release (MR) and fast test MR formulations of dextromethorphan hydrobromide were determined. Cumulative percentage drug release at various time intervals were calculated and are presented in Tables 59 & 60 and in Figures 37 & 38. The similarity factor ($f_2$) was calculated and is presented in Table 61.

When dissolution tests were performed at pH 1.2 buffer, pH 4.5, pH 5.5, buffer and pH 6.8 at 50 and 75 rpm, the release of the dextromethorphan hydrobromide was found to be almost indistinguishable between the slow and fast formulations. The $f_2$ value for pH 1.2 buffer, pH 4.5, pH 5.5, buffer and pH 6.8 at 50 rpm was 60.61,42.06, 60.72 and 48.79, respectively, whereas at 75 rpm, the $f_2$ value was 61.49, 59.62, 43.64 and 52.02, respectively. The higher $f_2$ values (more than 50) confirms that these dissolution mediums are indistinguishable and ensures sameness or equivalence between the two dissolution profiles and hence not considered for the present study.

The best discrimination was achieved at pH 7.4 buffer at 50 rpm as well as 75 rpm. The $f_2$ value for pH 7.4 buffer at 50 rpm was 34.44 whereas at 75 rpm, the $f_2$ value was 41.49. The associated $f_2$ metric, an $f_2$ value below 50 suggests that the two dissolution profiles are dissimilar and reveals pH 7.4 buffer at 75 rpm was more discriminating dissolution mediums and hence selected for IVIVC model development.

Level A correlation was developed by a two-stage procedure, deconvolution followed by comparison of the percent dissolved versus the percent absorbed data for both the slow and fast formulations. The *in vitro- in vivo* correlation plot was constructed using percentage of drug dissolved at pH 7.4 buffer dissolution media at both 50 and 75 rpm versus the percentage of drug absorbed. The slope of the best-fit line was examined using linear regression

analysis and coefficient of correlation ($r^2$). The slope and intercept values were calculated and are presented in Tables 62 & 63 and in Figures 39 & 40. The correlation coefficient ($r^2$) for pH 7.4 buffer at 50 rpm and 75 rpm was 0.9177 and 0.9604, respectively. A good linear regression relationship was thus observed when the dissolution studies were carried out in pH 7.4 buffer at 75 rpm and hence this was selected for further analysis.

The dissolution rate constants were determined from percentage drug released versus the square root of time. The slope of the best-fit line for the semi-log treatment of this data was taken as the first order rate constant for absorption. Linear regression analysis was applied to the *in vitro- in vivo* correlation plots and coefficient of correlation ($r^2$), slope and intercept values were calculated and are presented in Figures 41 - 43. The correlation coefficient ($r^2$) for pH 7.4 buffer at 75 was 0.9875. A good linear regression relationship was thus observed using pH 7.4 buffer as dissolution medium at 75 rpm and hence this was selected as the dissolution media of choice.

#### 2.9.2.1 Internal Validation

The predictability of the IVIVC was examined by using the mean *in vitro* dissolution data and mean *in vivo* pharmacokinetics of the selected modified release formulations. The mean *in vitro* dissolution rate constants were correlated to the mean absorption rate constants for the modified release formulations. These two data points, along with the zero-zero intercept were used to calculate the expected absorption rate constants.

The prediction of plasma concentration was calculated. From this, percentage prediction errors for $C_{max}$ and AUC were calculated and are presented in Tables 64 & 65 and in Figures 44 & 45. The $C_{max}$ prediction errors for both the slow and fast formulations were found to be -6.98 and -8.55, respectively. These

values were very close to the observed mean values. The AUC prediction error was 7.76 and 8.82 % for slow and fast formulations, respectively.

The $C_{max}$ and AUC prediction error was within the specified limit and hence, the IVIVC is considered as validated.

**Table 5 Granule properties of the different formulations of Ondansetron hydrochloride**

| Formulation F[a] | Angle of repose (θ°) | LBD[b] (g/ml) | TBD[c] (g/ml) | Carr`s Index (%) |
|---|---|---|---|---|
| $F_1$ | 37.23 | 0.51 | 0.58 | 13.06 |
| $F_2$ | 38.30 | 0.30 | 0.35 | 12.89 |
| $F_3$ | 36.50 | 0.36 | 0.42 | 14.29 |
| $F_4$ | 38.65 | 0.28 | 0.34 | 16.52 |
| $F_5$ | 36.26 | 0.29 | 0.36 | 19.44 |
| $F_6$ | 37.88 | 0.35 | 0.42 | 16.67 |
| $F_7$ | 32.45 | 0.38 | 0.47 | 19.15 |
| $F_8$ | 31.63 | 0.34 | 0.41 | 17.07 |
| $F_9$ | 33.42 | 0.42 | 0.48 | 12.50 |
| $F_{10}$ | 32.76 | 0.43 | 0.48 | 10.42 |
| $F_{11}$ | 31.25 | 0.39 | 0.50 | 22.00 |
| $F_{12}$ | 32.65 | 0.42 | 0.53 | 20.75 |
| $F_{13}$ | 34.21 | 0.43 | 0.48 | 10.42 |
| $F_{14}$ | 27.66 | 0.49 | 0.58 | 15.52 |
| $F_{15}$ | 28.73 | 0.47 | 0.55 | 14.55 |
| $F_{16}$ | 29.98 | 0.44 | 0.57 | 22.81 |
| $F_{17}$ | 31.54 | 0.45 | 0.56 | 19.64 |

[a]Code of formulations,

[b]Loose Bulk Density,

[c]Tapped Bulk Density.

**Table 6 Granule properties of the different formulations of Dextromethorphan hydrobromide**

| Formulation F[a] | Angle of repose (θ°) | LBD[b] (g/ml) | TBD[c] (g/ml) | Carr`s Index (%) |
|---|---|---|---|---|
| $F_1$ | 25.11 | 0.28 | 0.34 | 16.52 |
| $F_2$ | 22.97 | 0.27 | 0.34 | 21.30 |
| $F_3$ | 24.28 | 0.26 | 0.37 | 30.05 |
| $F_4$ | 23.88 | 0.29 | 0.42 | 31.65 |
| $F_5$ | 23.96 | 0.27 | 0.37 | 26.85 |
| $F_6$ | 24.11 | 0.27 | 0.39 | 29.90 |
| $F_7$ | 24.15 | 0.30 | 0.43 | 29.58 |
| $F_8$ | 23.76 | 0.26 | 0.35 | 26.93 |
| $F_9$ | 27.72 | 0.31 | 0.40 | 22.14 |
| $F_{10}$ | 25.55 | 0.28 | 0.31 | 8.85 |
| $F_{11}$ | 24.30 | 0.33 | 0.46 | 27.29 |
| $F_{12}$ | 25.38 | 0.35 | 0.42 | 18.01 |
| $F_{13}$ | 22.30 | 0.25 | 0.32 | 21.56 |
| $F_{14}$ | 25.18 | 0.28 | 0.41 | 31.07 |
| $F_{15}$ | 24.23 | 0.32 | 0.46 | 29.76 |
| $F_{16}$ | 20.41 | 0.36 | 0.44 | 18.68 |
| $F_{17}$ | 27.72 | 0.32 | 0.44 | 27.44 |
| $F_{18}$ | 25.29 | 0.35 | 0.51 | 32.62 |

[a]Code of formulations,

[b]Loose Bulk Density,

[c]Tapped Bulk Density.

**Figure 4a Differential Scanning Calorimetry (DSC) spectra of Ondansetron hydrochloride**

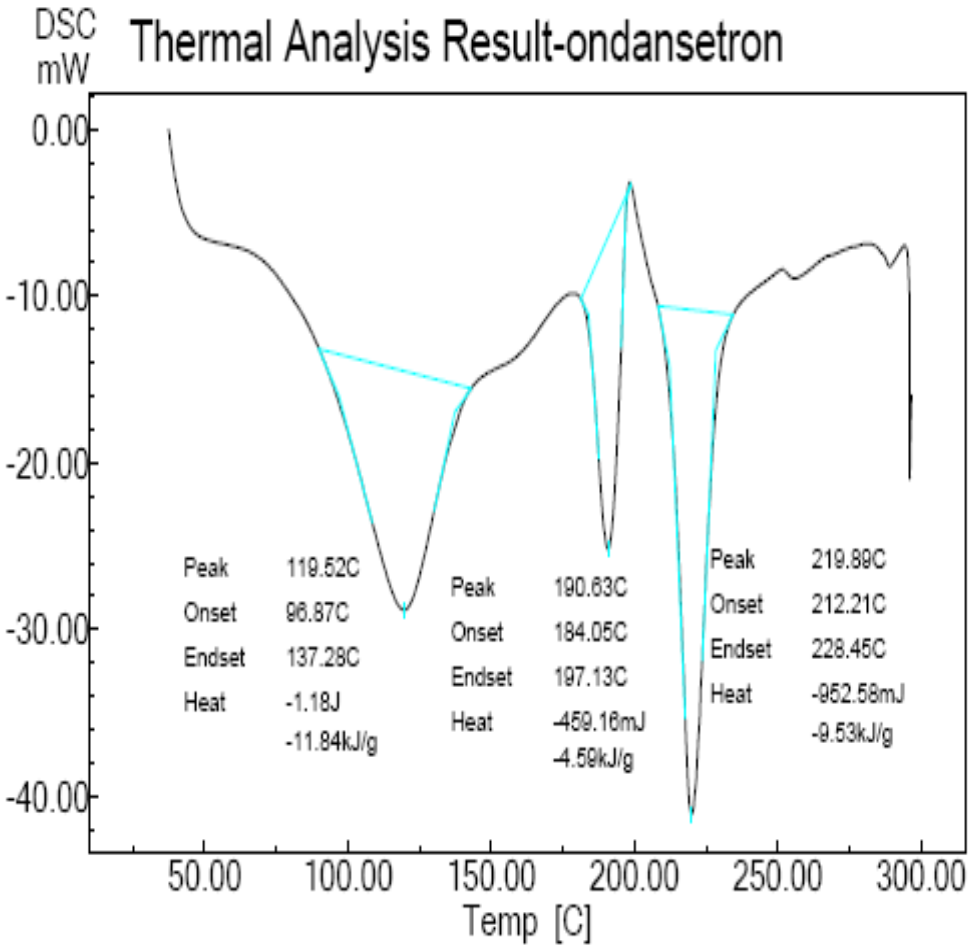


**Figure 4b Differential Scanning Calorimetry (DSC) spectra of Ondansetron hydrochloride and polymer**

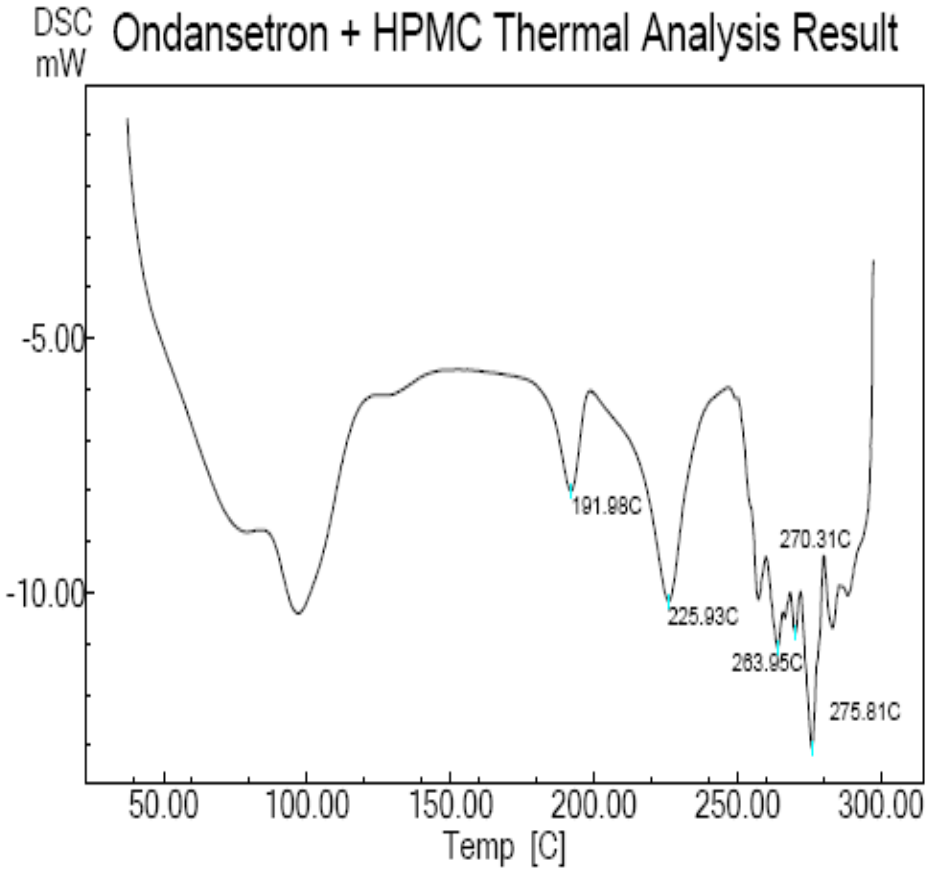


**Figure 5a Differential Scanning Calorimetry (DSC) spectra of Dextromethorphan hydrobromide**

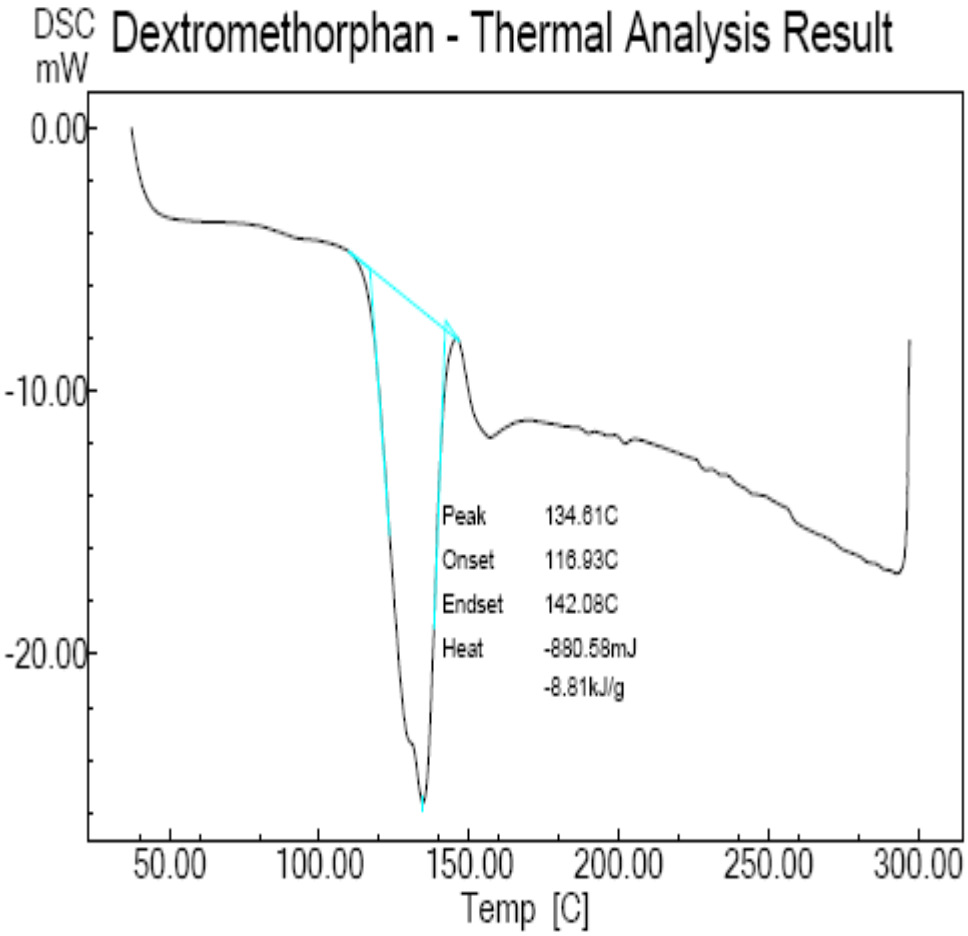


**Figure 5b Differential Scanning Calorimetry (DSC) spectra of Dextromethorphan hydrobromide and polymer**

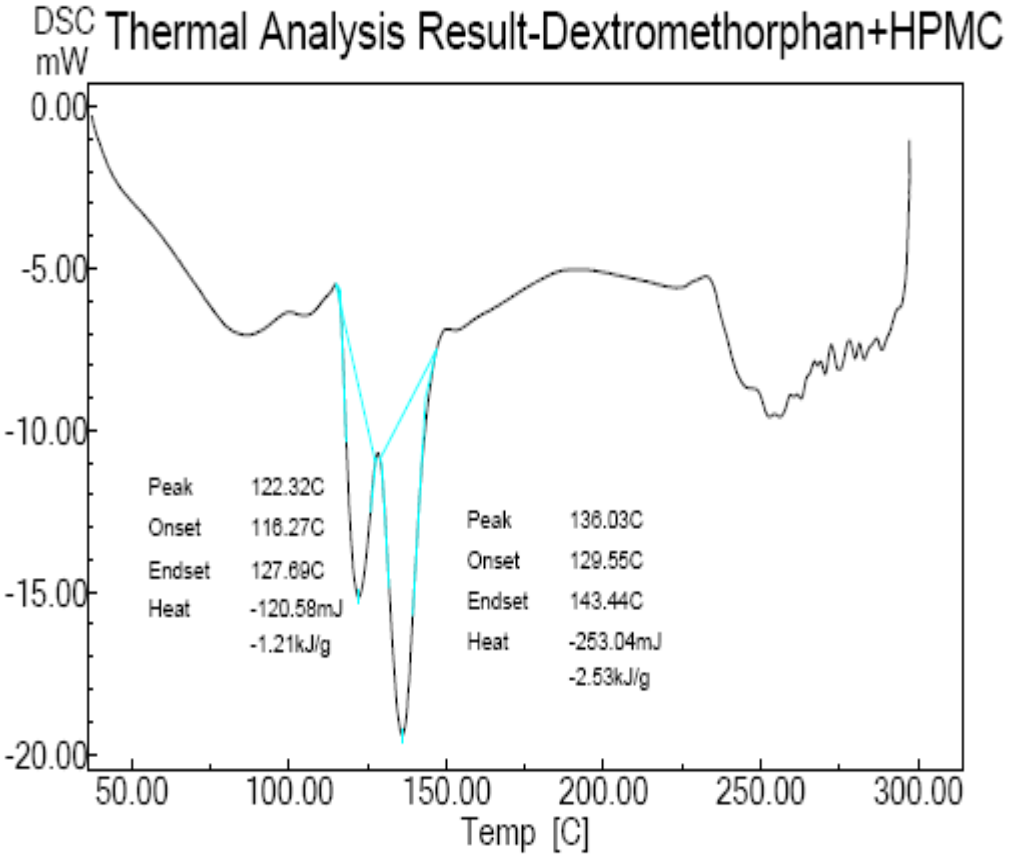


**Figure 6a Infrared (IR) spectra of Ondansetron hydrochloride**

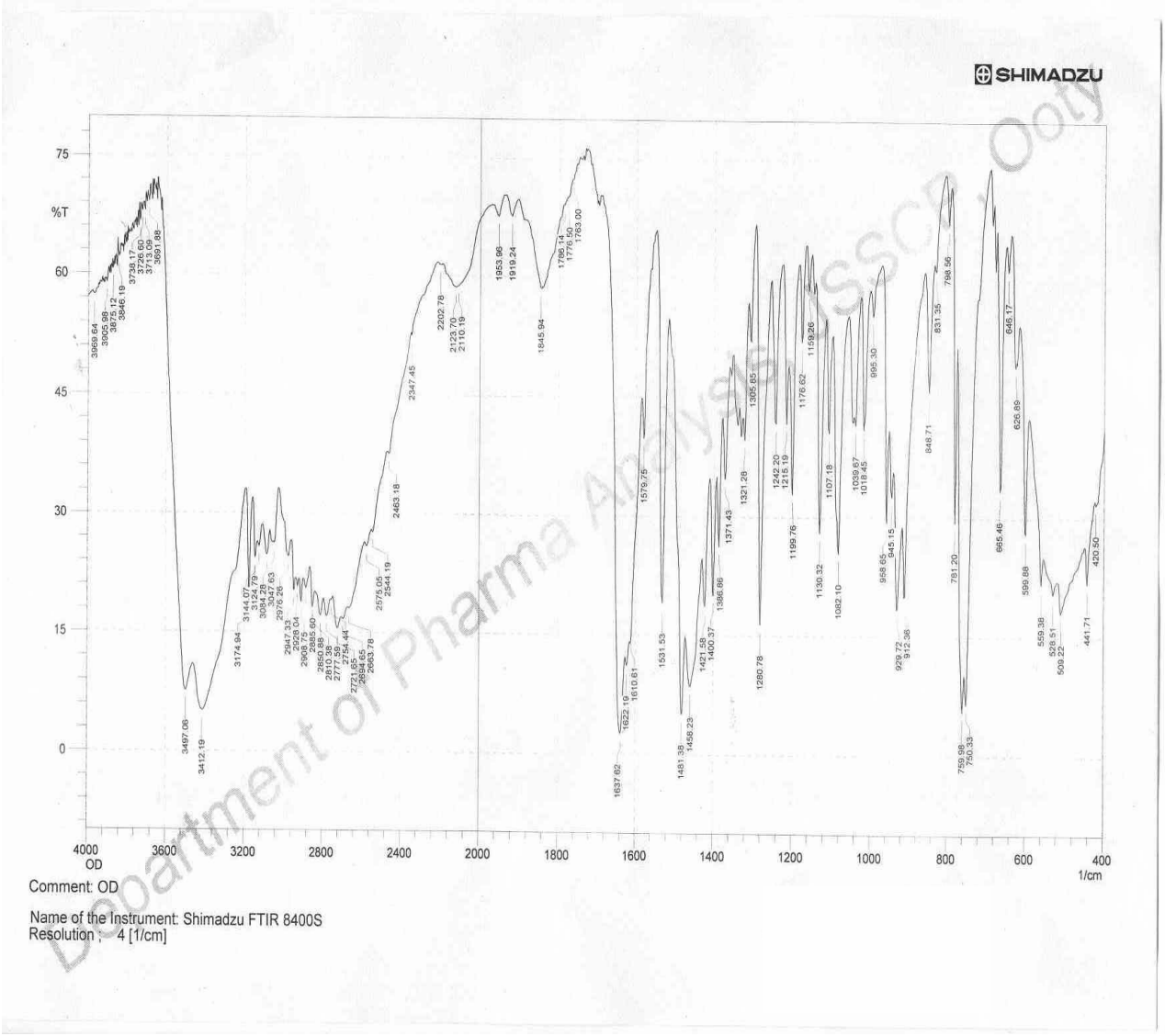


**Figure 6b Infrared (IR) spectra of Ondansetron hydrochloride and polymer**

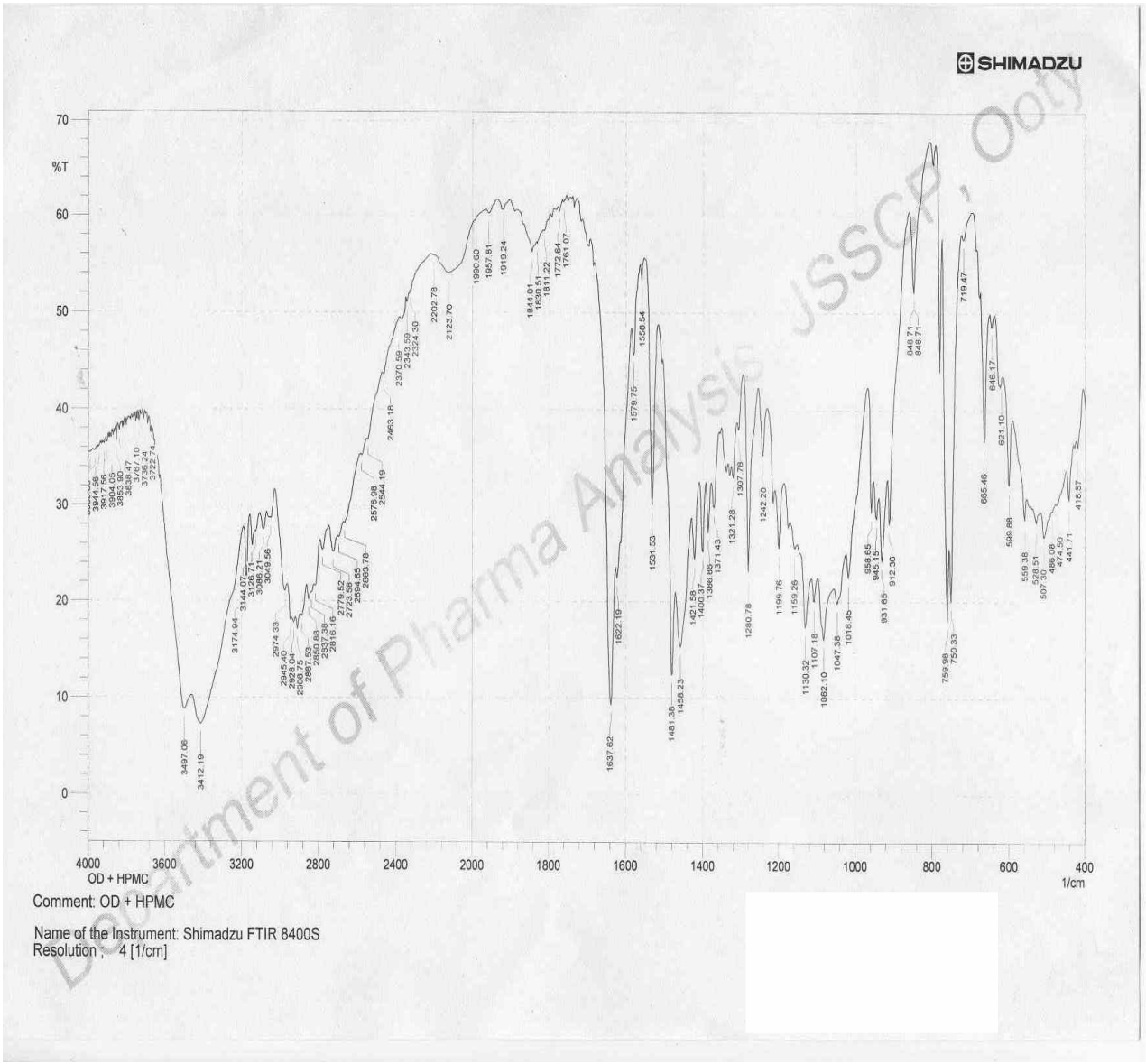


**Figure 7a Infrared (IR) spectra of Dextromethorphan hydrobromide**

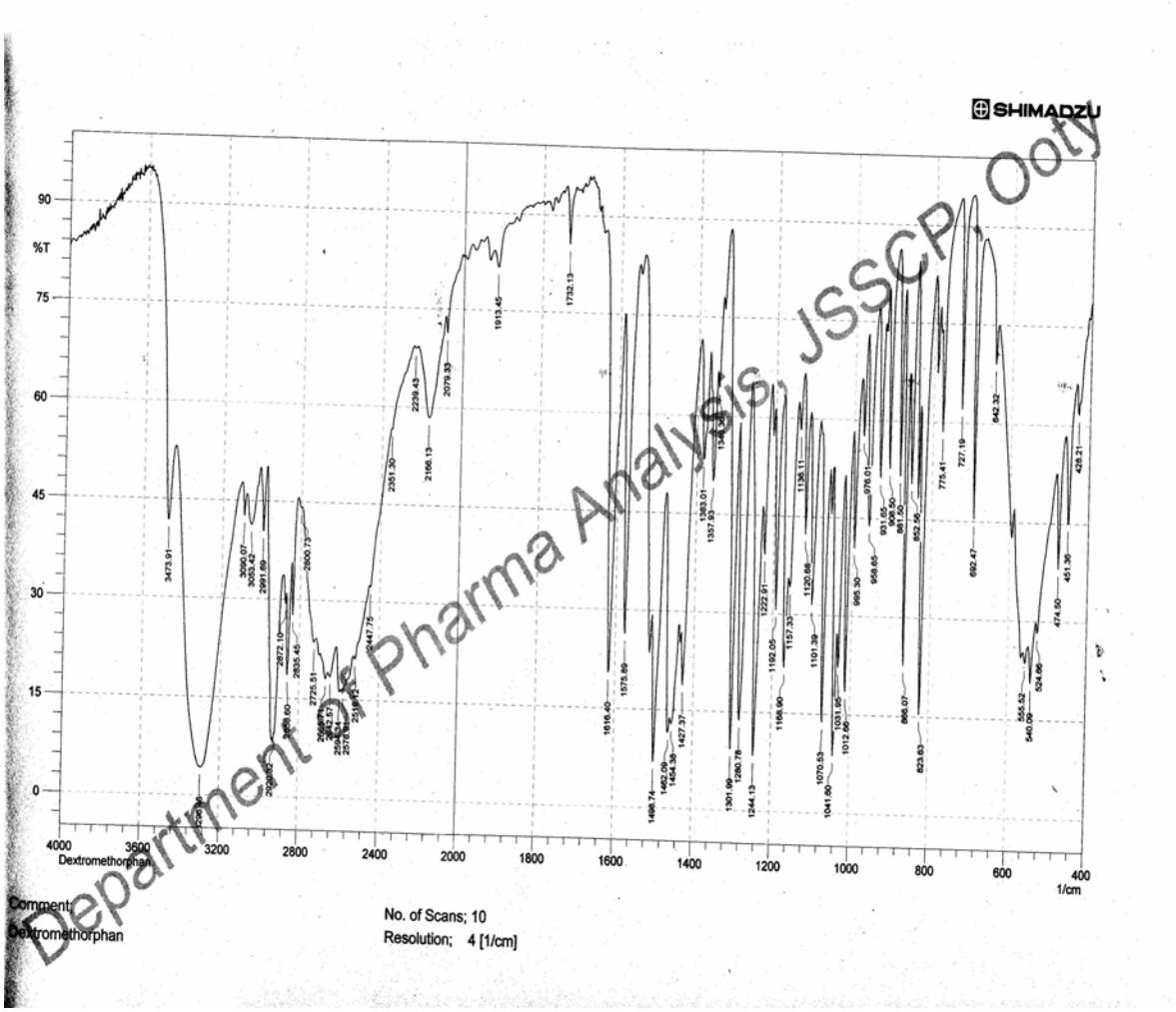


**Figure 7b Infrared (IR) spectra of Dextromethorphan hydrobromide and polymer**

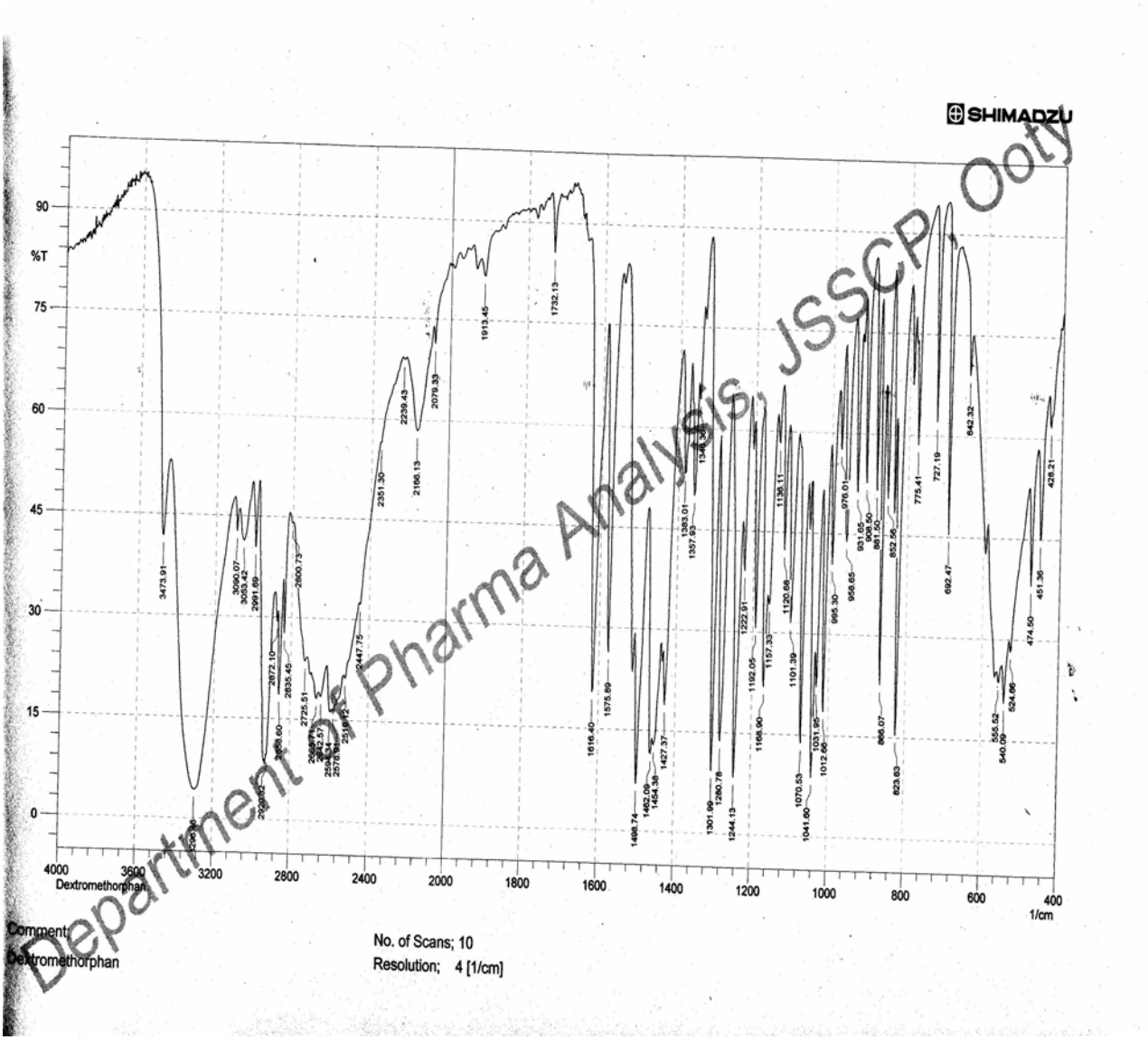


**Table 7 Comparison of the physical properties of the matrix tablets containing Ondansetron**

| Formulation F[a] | Hardness (kg/cm²)[b] | Thickness (mm)[b] | Weight (g)[b] | Friability (%)[b] |
|---|---|---|---|---|
| $F_1$ | 5.00±0.35 | 3.50±0.07 | 0.1028±0.002 | 0.42± 0.01 |
| $F_2$ | 4.98±0.01 | 3.52±0.07 | 0.1039±0.001 | 0.41± 0.02 |
| $F_3$ | 4.95±0.01 | 3.48±0.16 | 0.1025±0.003 | 0.40± 0.02 |
| $F_4$ | 4.92±0.07 | 3.51±0.14 | 0.1020±0.001 | 0.43± 0.02 |
| $F_5$ | 4.99±0.26 | 3.49±0.18 | 0.1027±0.000 | 0.39± 0.02 |
| $F_6$ | 5.01±0.03 | 3.43±0.27 | 0.1015±0.000 | 0.38± 0.02 |
| $F_7$ | 5.04±0.27 | 3.42±0.15 | 0.1021±0.000 | 0.43± 0.01 |
| $F_8$ | 4.98±0.06 | 3.44±0.12 | 0.1020±0.001 | 0.45± 0.01 |
| $F_9$ | 4.90±0.13 | 3.54±0.09 | 0.1036±0.000 | 0.39± 0.04 |
| $F_{10}$ | 4.91±0.39 | 3.53±0.16 | 0.1018±0.000 | 0.35± 0.03 |
| $F_{11}$ | 4.99±0.02 | 3.48±0.18 | 0.1022±0.000 | 0.44± 0.02 |
| $F_{12}$ | 4.98±0.03 | 3.55±0.06 | 0.1020±0.002 | 0.36± 0.03 |
| $F_{13}$ | 5.06±0.25 | 3.53±0.02 | 0.1047±0.000 | 0.35± 0.03 |
| $F_{14}$ | 5.01±0.12 | 3.82±0.07 | 0.1038±0.001 | 0.36± 0.02 |
| $F_{15}$ | 4.94±0.15 | 3.69±0.06 | 0.1035±0.000 | 0.37± 0.05 |
| $F_{16}$ | 4.95±0.18 | 3.84±0.08 | 0.1040±0.007 | 0.45± 0.05 |
| $F_{17}$ | 4.91±0.32 | 3.85±0.17 | 0.1062±0.006 | 0.46± 0.02 |

[a] Code of formulations

[b] Results represents the mean of replicate determination with the standard deviation given in parenthesis

**Table 8 Comparison of the physical properties of the matrix tablets containing Dextromethorphan**

| Formulation F[a] | Hardness (kg/cm²)[b] | Thickness (mm)[b] | Weight (g)[b] | Friability (%)[b] |
|---|---|---|---|---|
| $F_1$ | 5.00±0.04 | 3.56±0.03 | 0.1980±0.002 | 1.21± 0.15 |
| $F_2$ | 4.98±0.06 | 3.56±0.08 | 0.2070±0.004 | 0.13± 0.02 |
| $F_3$ | 5.02±0.05 | 3.65±0.06 | 0.2020±0.002 | 0.12± 0.06 |
| $F_4$ | 4.55±0.20 | 3.71±0.03 | 0.2019±0.003 | 0.06± 0.01 |
| $F_5$ | 5.17±0.28 | 3.74±0.03 | 0.2037±0.003 | 0.05± 0.02 |
| $F_6$ | 5.37±0.27 | 3.80±0.11 | 0.2090±0.002 | 0.11± 0.06 |
| $F_7$ | 4.51±0.41 | 3.73±0.04 | 0.2006±0.004 | 0.03± 0.00 |
| $F_8$ | 5.55±0.27 | 3.70±0.03 | 0.2021±0.005 | 0.49± 0.07 |
| $F_9$ | 6.00±0.20 | 3.84±0.06 | 0.2043±0.007 | 0.01± 0.00 |
| $F_{10}$ | 5.50±0.29 | 3.79±0.03 | 0.2014±0.008 | 0.06± 0.03 |
| $F_{11}$ | 6.00±0.17 | 3.88±0.02 | 0.2048±0.007 | 0.05± 0.01 |
| $F_{12}$ | 5.31±0.36 | 4.01±0.02 | 0.2074±0.002 | 0.06± 0.01 |
| $F_{13}$ | 5.13±0.12 | 3.78±0.04 | 0.2084±0.003 | 0.08± 0.02 |
| $F_{14}$ | 4.83±0.14 | 3.82±0.07 | 0.2110±0.005 | 0.24± 0.05 |
| $F_{15}$ | 4.62±0.24 | 3.69±0.06 | 0.2031±0.005 | 0.08± 0.01 |
| $F_{16}$ | 5.00±0.08 | 3.84±0.08 | 0.2019±0.007 | 0.09± 0.01 |
| $F_{17}$ | 4.75±0.21 | 3.85±0.17 | 0.2062±0.006 | 0.10± 0.02 |
| $F_{18}$ | 5.09±0.39 | 4.00±0.05 | 0.2173±0.003 | 0.18± 0.07 |

[a] Code of formulations

[b] Results represents the mean of replicate determination with the standard deviation given in parenthesis

**Figure 8 Release profiles of Ondansetron from HPMC (polymer) containing Formulations ($F_1$-$F_6$)**

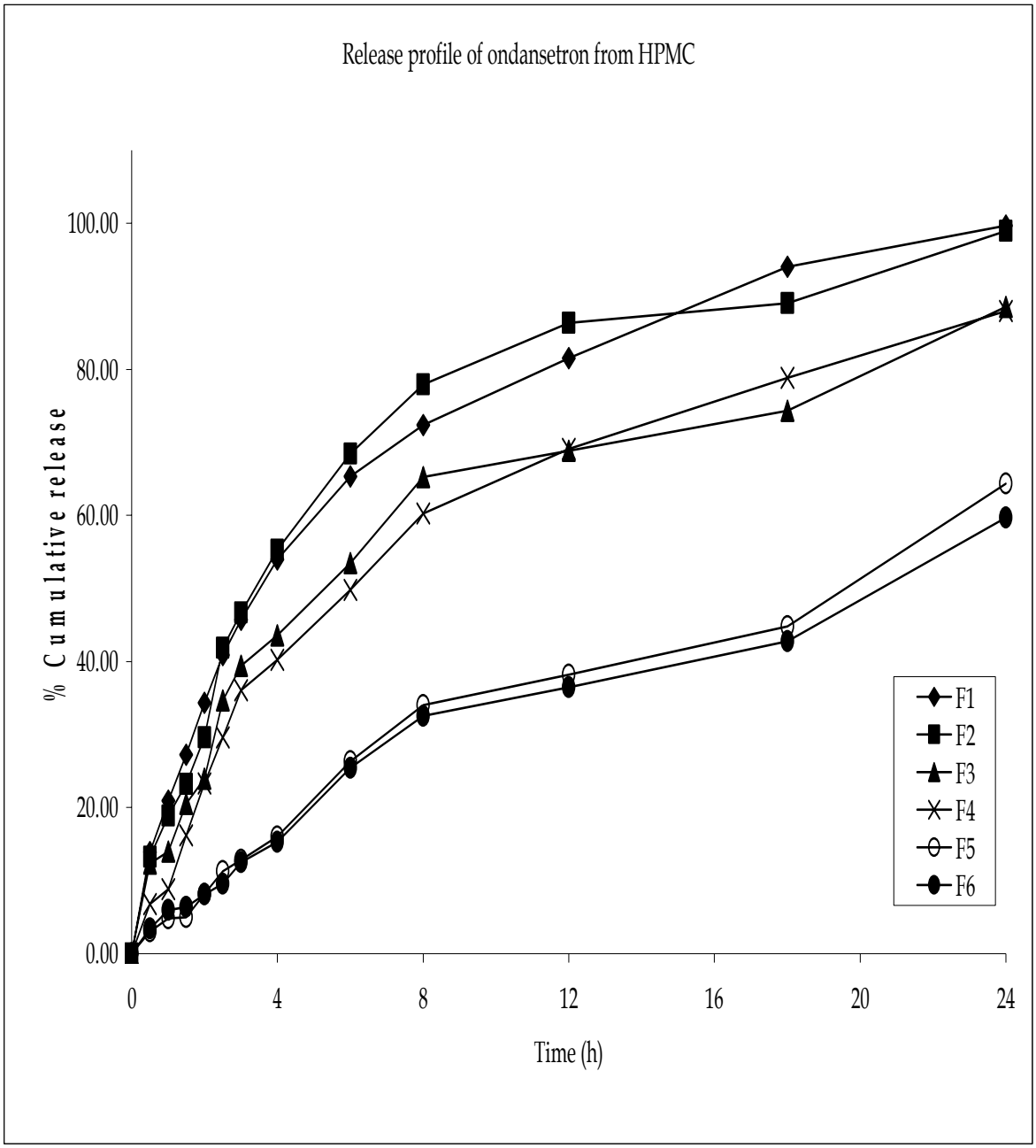


**Figure 9 Release profiles of Ondansetron from Carbopol (polymer) containing Formulations ($F_7$-$F_{12}$)**

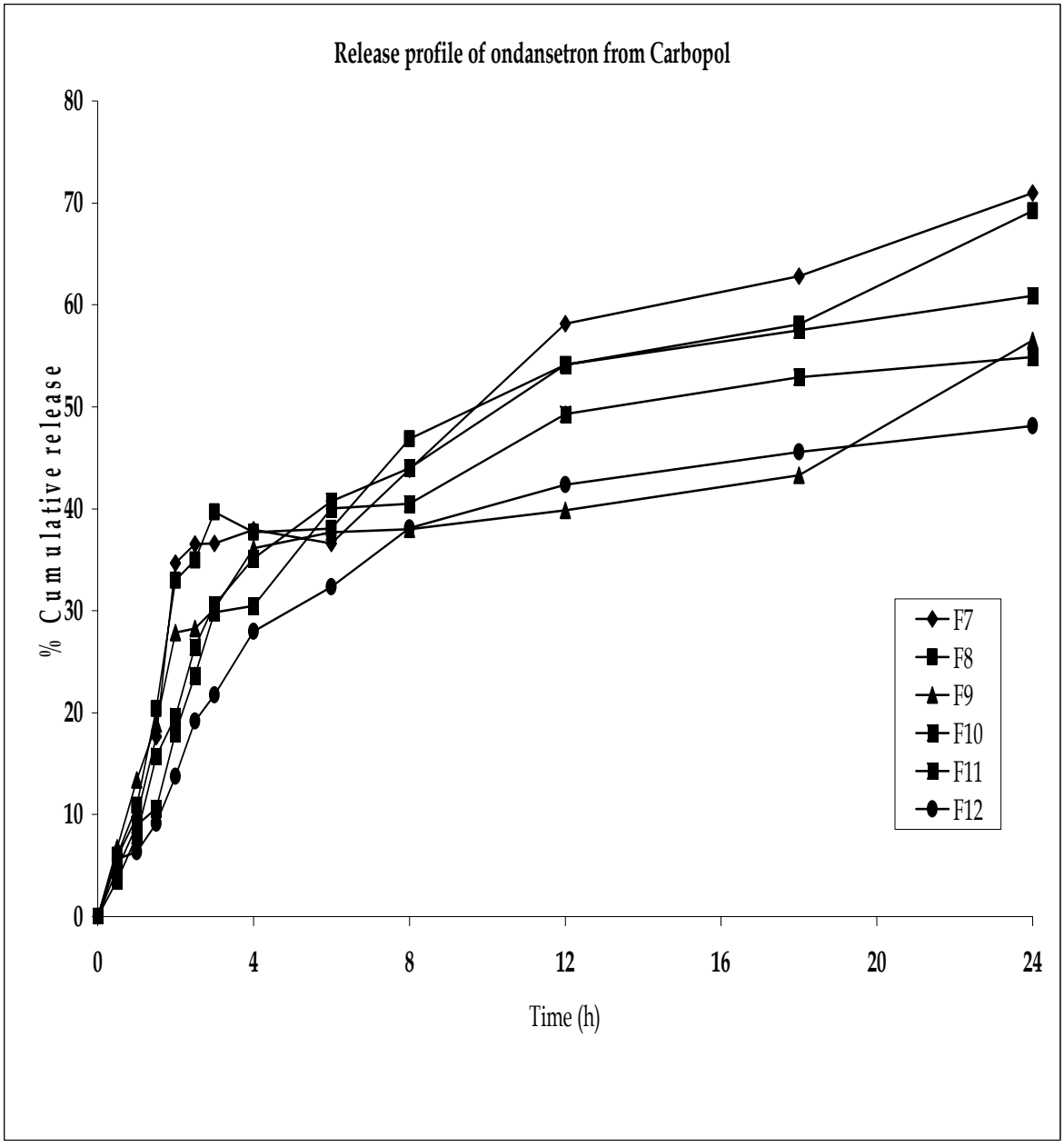


**Figure 10 Release profiles of Ondansetron from HPMC and Carbopol (polymers) containing Formulations ($F_{13}$-$F_{17}$)**

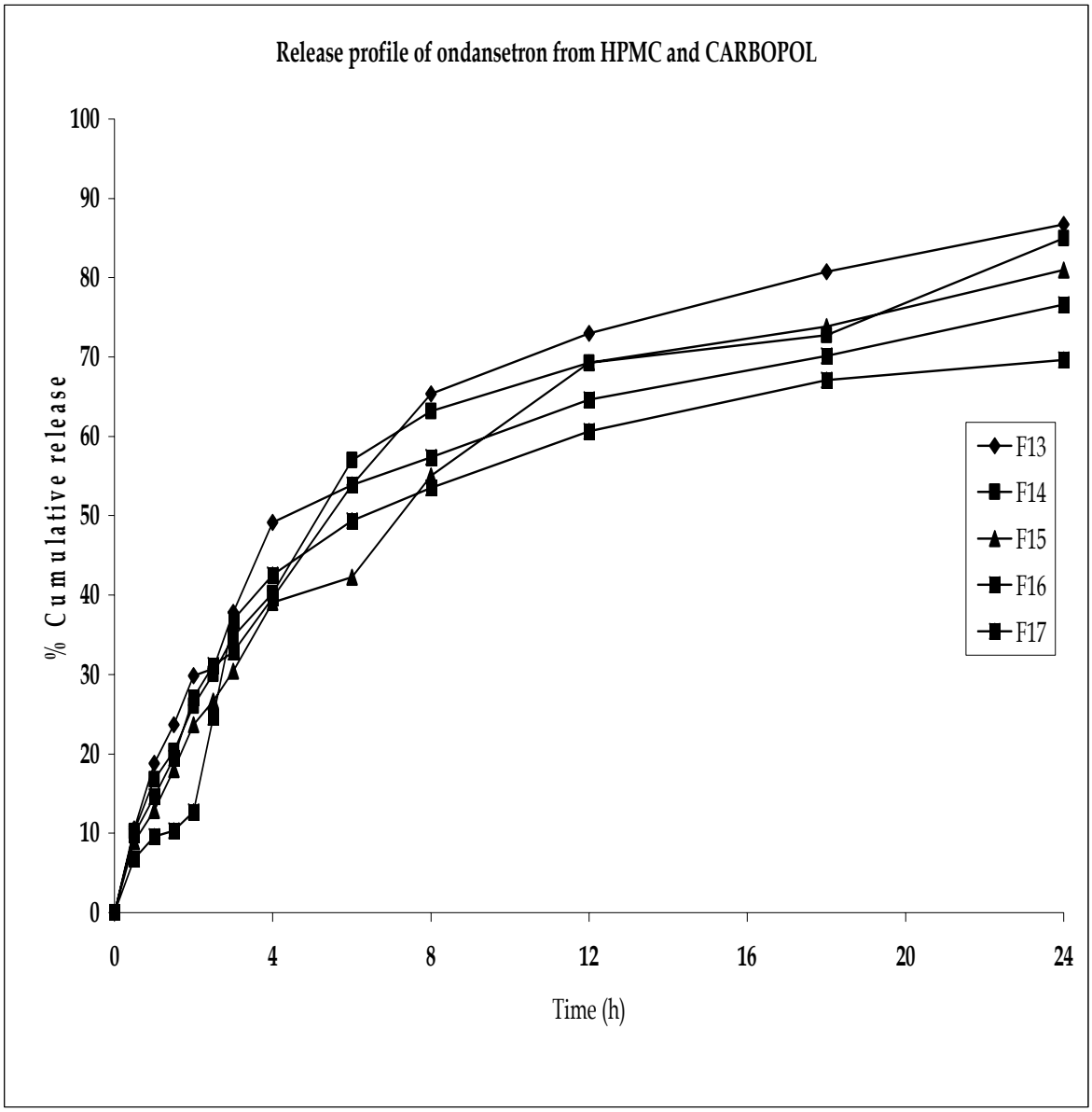


**Figure 11 Release profiles of Dextromethorphan from HPMC (polymer) containing Formulations ($F_1$-$F_6$)**

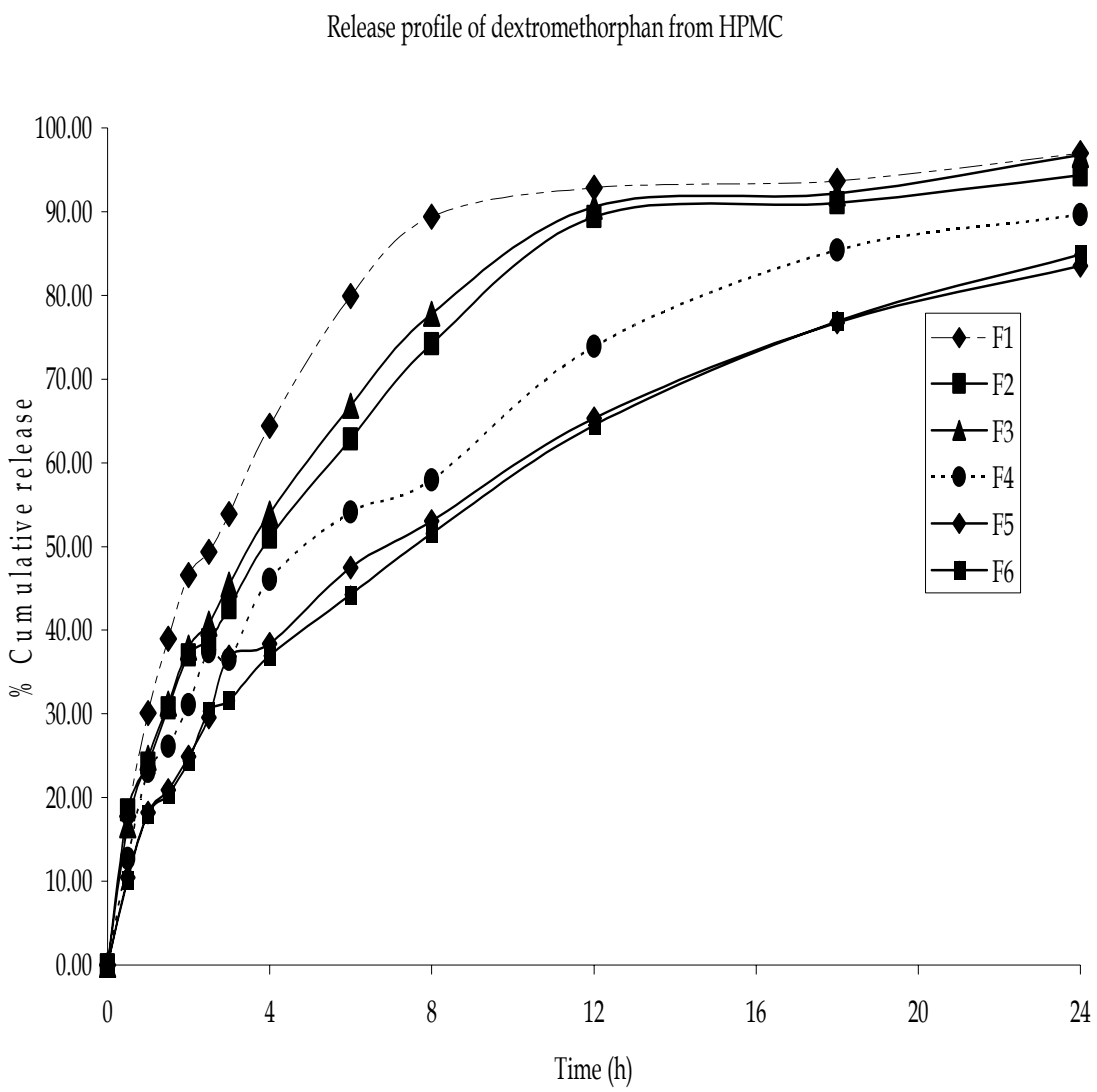


**Figure 12 Release profiles of Dextromethorphan from Carbopol (polymer) containing Formulations ($F_7$-$F_{12}$)**

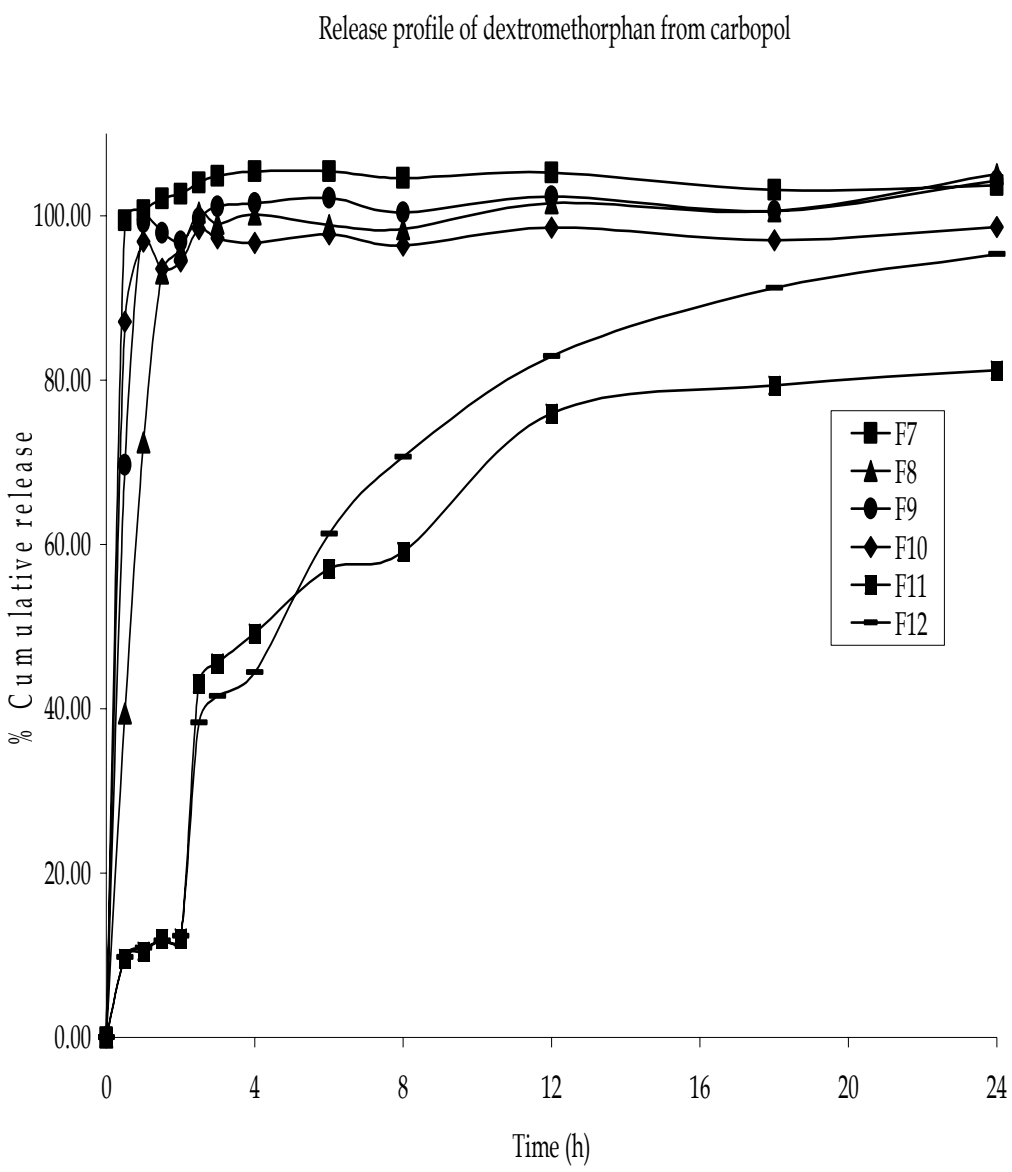


**Figure 13 Release profiles of Dextromethorphan from HPMC and Carbopol (polymers) containing Formulations ($F_{13}$-$F_{18}$)**

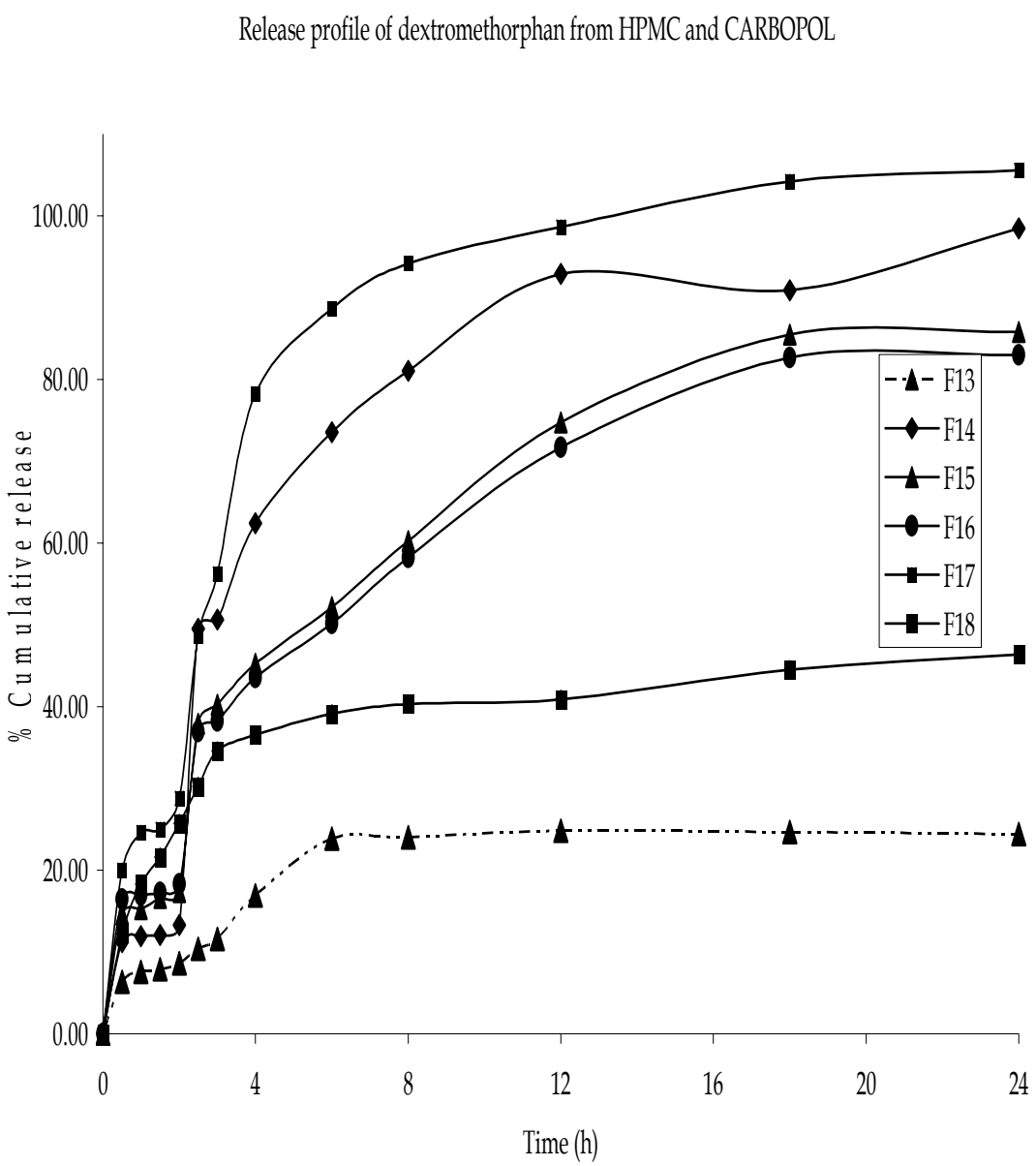


**Figure 14 Zero order chart of optimized Ondansetron formulations ($F_1$-$F_4$)**

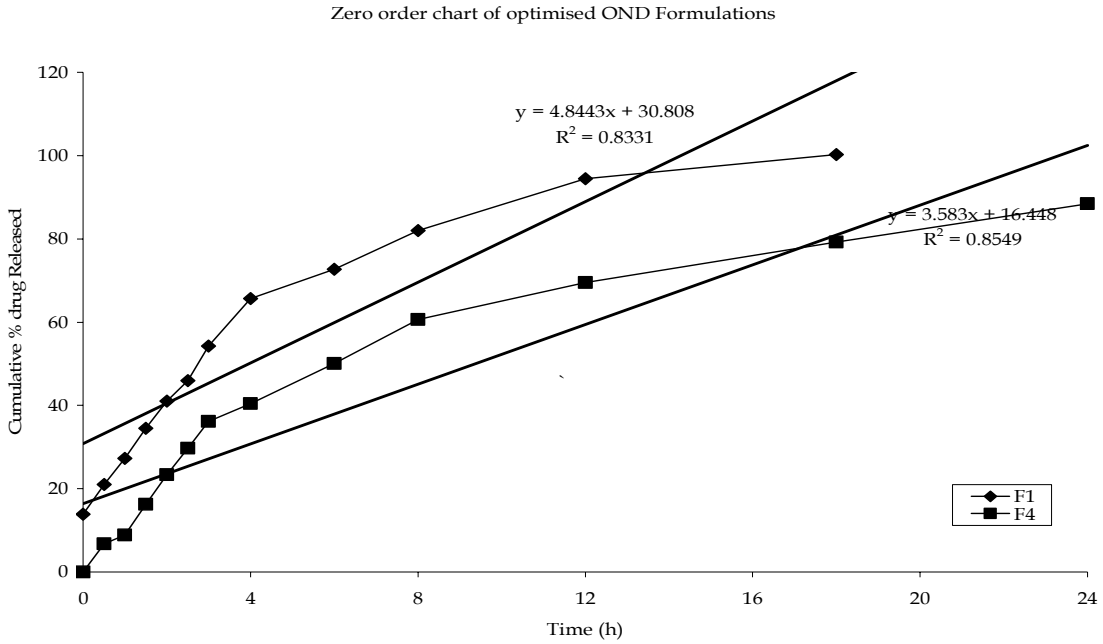



**Figure 15 First order chart of optimized Ondansetron formulations ($F_1$-$F_4$)**

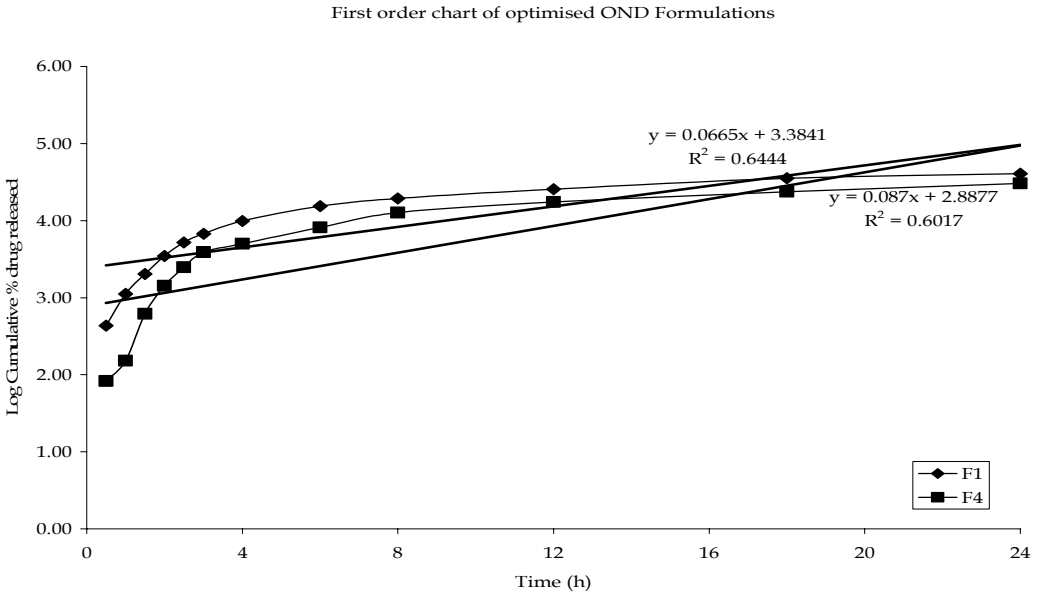


**Figure 16 Higuchi chart of optimized Ondansetron formulations ($F_1$-$F_4$)**

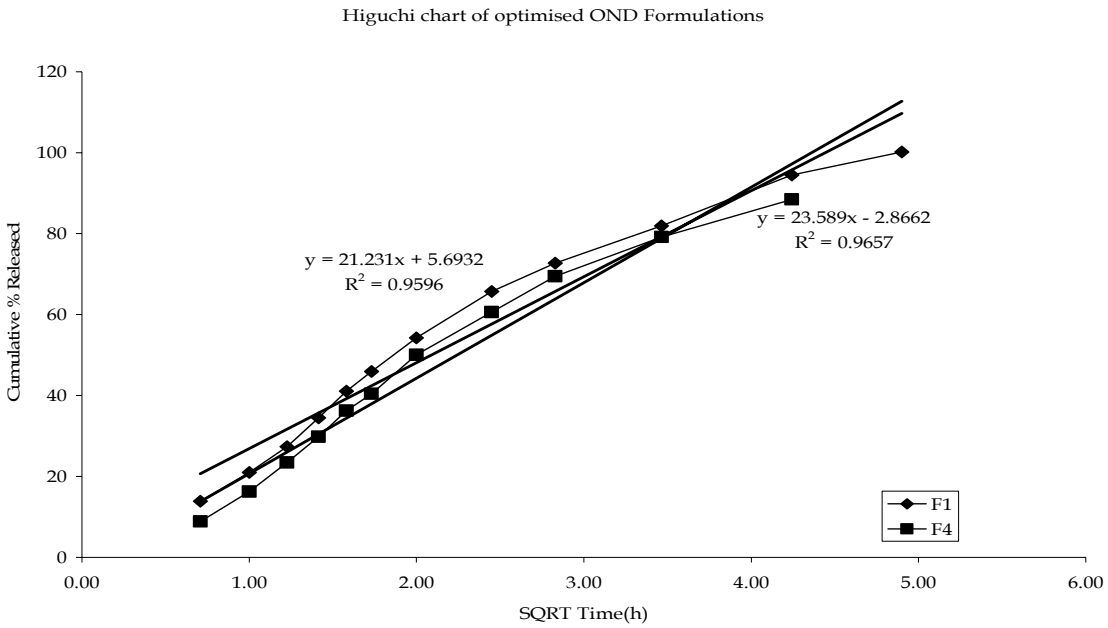



**Figure 17 Peppas chart of optimized Ondansetron formulations ($F_1$-$F_4$)**

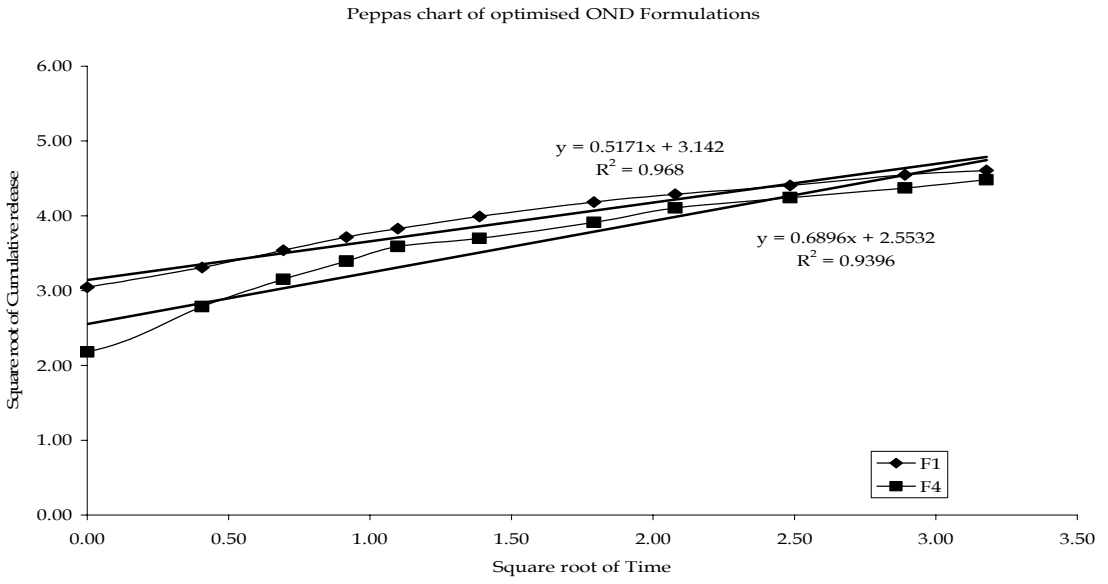


**Figure 18 Zero order chart of optimized Dextromethorphan formulation ($F_1$-$F_4$)**

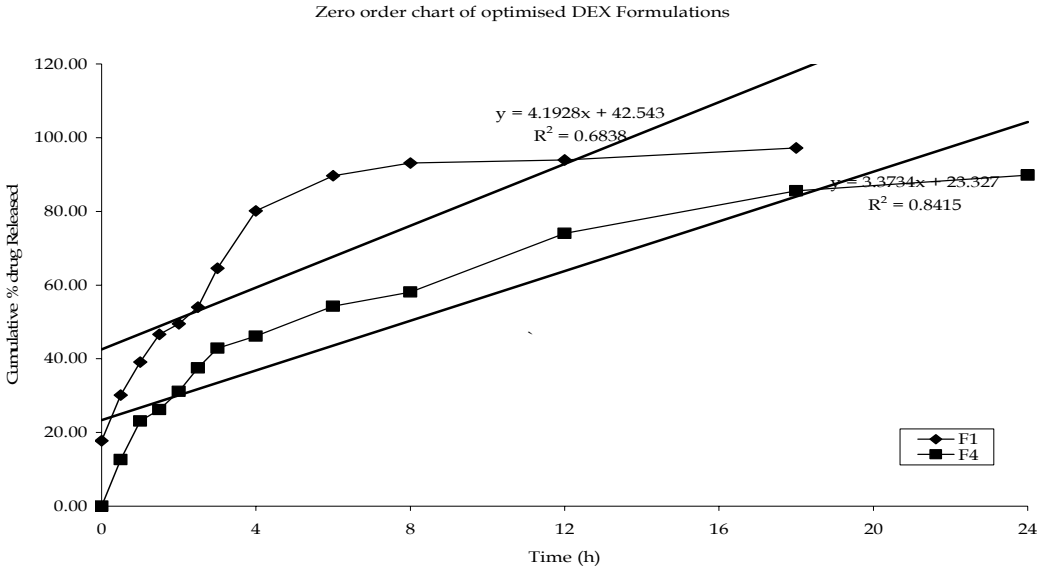



**Figure 19 First order chart of optimized Dextromethorphan formulations ($F_1$-$F_4$)**

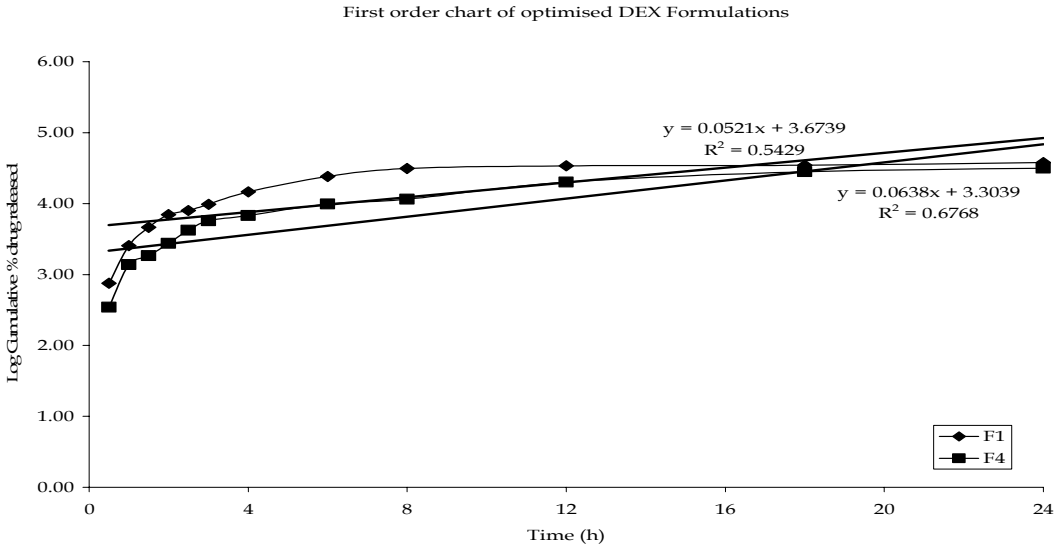


**Figure 20 Higuchi chart of optimized Dextromethorphan formulations ($F_1$-$F_4$)**

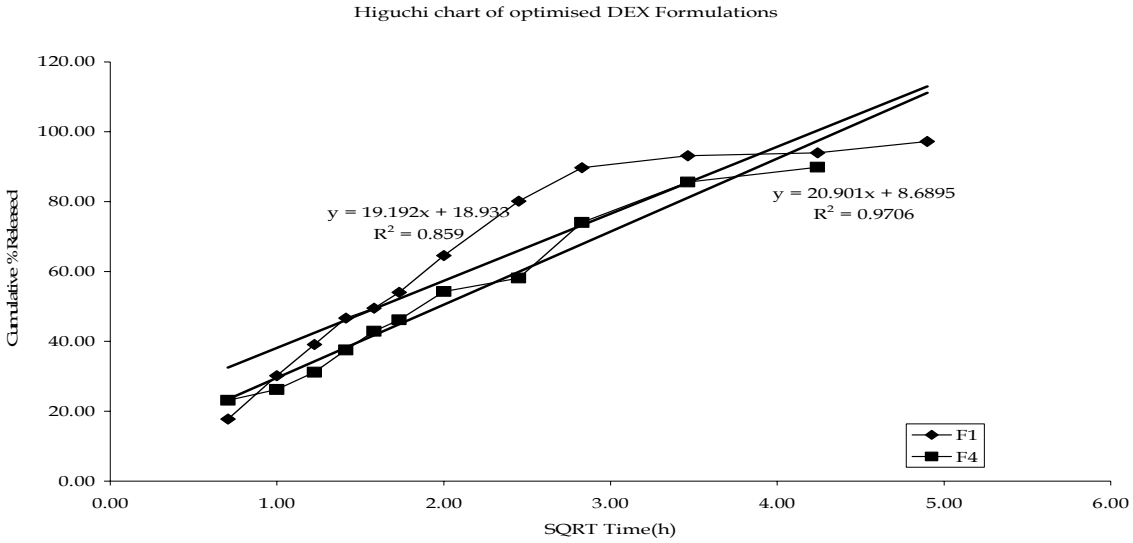



**Figure 21 Peppas chart of optimized Dextromethorphan formulations ($F_1$-$F_4$)**

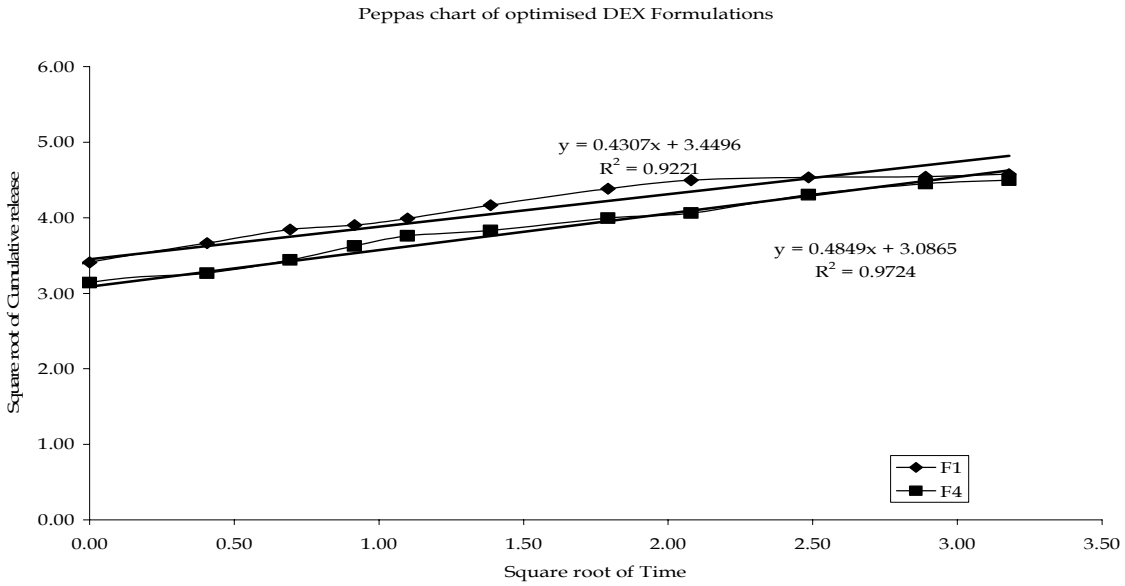


**Table 9 Stability data at the end of three months for Ondansetron hydrochloride**

| Parameter | Initial[a] | Real time[a] | Accelerated[a] |
|---|---|---|---|
| Thickness mm | 3.48±0.03 | 3.50±0.21 | 3.56±0.17 |
| Hardness ($kg/cm^2$) | 4.99±0.35 | 4.96±0.39 | 5.02±0.37 |
| Friability (%) | 0.42±0.02 | 0.44±0.04 | 0.46±0.06 |
| Drug content (%) | 98.64±1.16 | 98.84±0.81 | 98.44±1.00 |

**Table 10 Stability data at the end of three months for Dextromethorphan hydrobromide**

| Parameter | Initial[a] | Real time[a] | Accelerated[a] |
|---|---|---|---|
| Thickness mm | 3.61±0.20 | 3.65±0.34 | 3.67±0.25 |
| Hardness ($kg/cm^2$) | 4.98±0.03 | 4.99±0.02 | 5.01±0.05 |
| Friability (%) | 0.41±0.02 | 0.40±0.04 | 0.42±0.03 |
| Drug content (%) | 98.72±1.29 | 98.12±1.30 | 97.72±1.27 |

[a]Results represents the mean of replicate determination with the standard deviation given in parenthesis

**Table 11 Concentrations-response linearity data for Ondansetron**

| | Nominal Concentration (ng/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0.5** | **1.0** | **2.0** | **4.0** | **10.0** | **20.0** | **40.0** | **50.0** |
| 1 | 0.462 | 0.986 | 1.805 | 4.052 | 9.726 | 20.910 | 40.385 | 50.546 |
| 2 | 0.551 | 0.975 | 1.986 | 3.947 | 10.081 | 19.947 | 39.573 | 49.831 |
| 3 | 0.492 | 0.933 | 1.867 | 3.892 | 9.908 | 20.097 | 38.154 | 48.384 |
| 4 | 0.506 | 0.873 | 2.194 | 4.184 | 9.653 | 19.231 | 39.894 | 50.506 |
| Mean | 0.5028 | 0.9418 | 1.9630 | 4.0188 | 9.8420 | 20.0463 | 39.5015 | 49.8168 |
| S.D (±) | 0.03703 | 0.05121 | 0.17134 | 0.12862 | 0.19205 | 0.68875 | 0.95838 | 1.00993 |
| C.V (%) | 7.37 | 5.44 | 8.73 | 3.20 | 1.95 | 3.44 | 2.43 | 2.03 |
| % Nominal | 100.55 | 94.18 | 98.15 | 100.47 | 98.42 | 100.23 | 98.75 | 99.63 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

## Table 12a Within Batch Precision and Accuracy for Ondansetron

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | **0.500** | **1.000** | **10.000** | **40.000** |
| 1 | 0.506 | 1.050 | 10.188 | 39.719 |
| 2 | 0.598 | 1.123 | 9.647 | 39.728 |
| 3 | 0.487 | 1.165 | 9.247 | 40.509 |
| 4 | 0.510 | 1.009 | 9.894 | 40.207 |
| 5 | 0.531 | 1.023 | 10.121 | 41.109 |
| Mean | 0.5264 | 1.0740 | 9.8194 | 40.2544 |
| S.D (±) | 0.04305 | 0.06724 | 0.38398 | 0.58334 |
| C.V (%) | 8.18 | 6.26 | 3.91 | 1.45 |
| % Nominal | 105.29 | 107.40 | 98.19 | 100.64 |
| N | 5 | 5 | 5 | 5 |
| 6 | 0.510 | 1.143 | 10.725 | 41.253 |
| 7 | 0.562 | 1.024 | 10.405 | 38.693 |
| 8 | 0.496 | 0.906 | 10.092 | 38.619 |
| 9 | 0.502 | 1.078 | 9.143 | 42.260 |
| 10 | 0.531 | 0.967 | 9.808 | 36.894 |
| Mean | 0.5202 | 1.0236 | 10.0346 | 39.5438 |
| S.D (±) | 0.02686 | 0.09253 | 0.60486 | 2.17369 |
| C.V (%) | 5.16 | 9.04 | 6.03 | 5.50 |
| % Nominal | 104.04 | 102.36 | 100.35 | 98.86 |
| N | 5 | 5 | 5 | 5 |
| 11 | 0.598 | 1.065 | 8.937 | 42.351 |
| 12 | 0.575 | 1.105 | 9.333 | 38.559 |
| 13 | 0.503 | 0.989 | 9.416 | 40.264 |
| 14 | 0.514 | 0.998 | 9.983 | 39.956 |
| 15 | 0.452 | 1.032 | 8.615 | 40.637 |
| Mean | 0.5284 | 1.0378 | 9.2568 | 40.3534 |
| S.D (±) | 0.05853 | 0.04810 | 0.51786 | 1.36534 |
| C.V (%) | 11.08 | 4.63 | 5.59 | 3.38 |
| % Nominal | 105.68 | 103.78 | 92.57 | 100.88 |
| N | 5 | 5 | 5 | 5 |
| 16 | 0.526 | 1.015 | 9.872 | 45.185 |
| 17 | 0.583 | 0.981 | 9.691 | 43.871 |
| 18 | 0.513 | 0.877 | 9.167 | 41.362 |
| 19 | 0.522 | 0.981 | 8.650 | 39.072 |
| 20 | 0.496 | 1.109 | 9.056 | 38.580 |
| Mean | 0.5280 | 0.9926 | 9.2872 | 41.6140 |
| S.D (±) | 0.03284 | 0.08319 | 0.49471 | 2.89726 |
| C.V (%) | 6.22 | 8.38 | 5.33 | 6.96 |
| % Nominal | 105.60 | 99.26 | 92.87 | 104.04 |
| N | 5 | 5 | 5 | 5 |

**Table 12b Between Batch / Inter day Precision and Accuracy for Ondansetron**

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | **0.500** | **1.000** | **10.000** | **40.000** |
| 1 | 0.506 | 1.050 | 10.188 | 39.719 |
| 2 | 0.598 | 1.123 | 9.647 | 39.728 |
| 3 | 0.487 | 1.165 | 9.247 | 40.509 |
| 4 | 0.510 | 1.009 | 9.894 | 40.207 |
| 5 | 0.531 | 1.023 | 10.121 | 41.109 |
| 6 | 0.510 | 1.143 | 10.725 | 41.253 |
| 7 | 0.562 | 1.024 | 10.405 | 38.693 |
| 8 | 0.496 | 0.906 | 10.092 | 38.619 |
| 9 | 0.502 | 1.078 | 9.143 | 42.260 |
| 10 | 0.531 | 0.967 | 9.808 | 36.894 |
| 11 | 0.598 | 1.065 | 8.937 | 42.351 |
| 12 | 0.575 | 1.105 | 9.333 | 38.559 |
| 13 | 0.503 | 0.989 | 9.416 | 40.264 |
| 14 | 0.514 | 0.998 | 9.983 | 39.956 |
| 15 | 0.452 | 1.032 | 8.615 | 40.637 |
| 16 | 0.526 | 1.015 | 9.872 | 45.185 |
| 17 | 0.583 | 0.981 | 9.691 | 43.871 |
| 18 | 0.513 | 0.877 | 9.167 | 41.362 |
| 19 | 0.522 | 0.981 | 8.650 | 39.072 |
| 20 | 0.496 | 1.109 | 9.056 | 38.580 |
| Mean | 0.5258 | 1.0320 | 9.5995 | 40.4414 |
| S.D (±) | 0.03875 | 0.07482 | 0.57893 | 1.95220 |
| C.V (%) | 7.37 | 7.25 | 6.03 | 4.83 |
| % Nominal | 105.15 | 103.20 | 96.00 | 101.10 |
| N | 20 | 20 | 20 | 20 |

**Table 12c Intra Day Precision and Accuracy for Ondansetron**

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | **0.500** | **1.000** | **10.000** | **40.000** |
| 1 | 0.506 | 1.050 | 10.188 | 39.719 |
| 2 | 0.598 | 1.123 | 9.647 | 39.728 |
| 3 | 0.487 | 1.165 | 9.247 | 40.509 |
| 4 | 0.510 | 1.009 | 9.894 | 40.207 |
| 5 | 0.531 | 1.023 | 10.121 | 41.109 |
| 6 | 0.510 | 1.143 | 10.725 | 41.253 |
| 7 | 0.562 | 1.024 | 10.405 | 38.693 |
| 8 | 0.496 | 0.906 | 10.092 | 38.619 |
| 9 | 0.502 | 1.078 | 9.143 | 42.260 |
| 10 | 0.531 | 0.967 | 9.808 | 36.894 |
| Mean | 0.5233 | 1.0488 | 9.9270 | 39.8991 |
| S.D (±) | 0.03399 | 0.08075 | 0.49091 | 1.54644 |
| C.V (%) | 6.49 | 7.70 | 4.95 | 3.88 |
| % Nominal | 104.66 | 104.88 | 99.27 | 99.75 |
| N | 10 | 10 | 10 | 10 |
| | Nominal Concentration (ng/ml) | | | |
| | LLOQ QC | LQC | MQC | HQC |
| | **0.500** | **1.000** | **10.000** | **40.000** |
| 11 | 0.598 | 1.065 | 8.937 | 42.351 |
| 12 | 0.575 | 1.105 | 9.333 | 38.559 |
| 13 | 0.503 | 0.989 | 9.416 | 40.264 |
| 14 | 0.514 | 0.998 | 9.983 | 39.956 |
| 15 | 0.452 | 1.032 | 8.615 | 40.637 |
| 16 | 0.526 | 1.015 | 9.872 | 45.185 |
| 17 | 0.583 | 0.981 | 9.691 | 43.871 |
| 18 | 0.513 | 0.877 | 9.167 | 41.362 |
| 19 | 0.522 | 0.981 | 8.650 | 39.072 |
| 20 | 0.496 | 1.109 | 9.056 | 38.580 |
| Mean | 0.5282 | 1.0152 | 9.2720 | 40.9837 |
| S.D (±) | 0.04475 | 0.06835 | 0.47772 | 2.23622 |
| C.V (%) | 8.47 | 6.73 | 5.15 | 5.46 |
| % Nominal | 105.64 | 101.52 | 92.72 | 102.46 |
| N | 10 | 10 | 10 | 10 |

**Table 13a Stock Stability of Ondansetron**

| S.No | Drug area | Drug area | Drug area |
|---|---|---|---|
| | 0 h | 3 h | 6 h |
| 1 | 285611 | 298018 | 276820 |
| 2 | 288631 | 294848 | 267093 |
| 3 | 282738 | 289721 | 251440 |
| 4 | 276264 | 273835 | 269484 |
| 5 | 292898 | 250825 | 268671 |
| Mean | 285228.4 | 281449.4 | 266701.6 |
| S.D | 6267.16 | 19482.64 | 9314.93 |
| C.V(%) | 2.20 | 6.92 | 3.49 |

**Table 13b 20°C Stability of Ondansetron in plasma**

| | Nominal Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **1.000** | **40.000** |
| | 0.986 | 36.683 |
| | 1.012 | 39.956 |
| | 0.961 | 38.837 |
| | 0.967 | 40.136 |
| | 0.993 | 38.729 |
| Mean | 0.9838 | 38.8682 |
| S.D | 0.02054 | 1.37714 |
| C.V(%) | 2.09 | 3.54 |
| % Nominal | 98.38 | 97.17 |
| N | 5 | 5 |

**Table 13c Short Term Room Temperature Stability of Ondansetron**

| | Nominal Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **1.000** | **40.000** |
| | 0.873 | 38.871 |
| | 0.937 | 39.686 |
| | 0.929 | 40.255 |
| | 1.014 | 39.065 |
| | 1.092 | 37.980 |
| Mean | 0.9690 | 39.1714 |
| S.D | 0.08514 | 0.86023 |
| C.V (%) | 8.79 | 2.20 |
| % Nominal | 96.90 | 97.93 |
| N | 5 | 5 |

**Table 13d Auto sampler Stability of Ondansetron in plasma**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **1.000** | **40.000** |
| | 1.052 | 38.859 |
| | 1.128 | 39.101 |
| | 0.876 | 37.253 |
| | 0.945 | 39.874 |
| | 0.982 | 38.906 |
| Mean | 0.9966 | 38.7986 |
| S.D | 0.09715 | 0.95551 |
| C.V (%) | 9.75 | 2.46 |
| % Nominal | 99.66 | 97.00 |
| N | 5 | 5 |

**Table 13e Freeze/thaw cycle stability**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **1.000** | **40.000** |
| | 1.092 | 38.517 |
| | 0.986 | 40.762 |
| | 0.943 | 40.979 |
| | 0.991 | 39.362 |
| | 1.107 | 39.369 |
| Mean | 1.0238 | 39.7978 |
| S.D (±) | 0.07178 | 1.04153 |
| C.V (%) | 7.01 | 2.62 |
| % Nominal | 102.38 | 99.49 |
| N | 5 | 5 |

**Table 13f Long term stability for four weeks**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **1.000** | **40.000** |
| | 0.991 | 37.685 |
| | 0.980 | 39.124 |
| | 0.906 | 40.025 |
| | 1.089 | 38.332 |
| | 0.967 | 40.657 |
| Mean | 0.9866 | 39.1646 |
| S.D (±) | 0.06601 | 1.20951 |
| C.V (%) | 6.69 | 3.09 |
| % Nominal | 98.66 | 97.91 |
| N | 5 | 5 |

**Table 14a Recovery study of Ondansetron**

| | LQC Response | | MQC Response | | HQC Response | |
|---|---|---|---|---|---|---|
| | Extracted | Unextracted | Extracted | Unextracted | Extracted | Unextracted |
| 1 | 9756 | 11020 | 189461 | 238354 | 386029 | 402464 |
| 2 | 7995 | 9831 | 199045 | 240392 | 368615 | 398109 |
| 3 | 8841 | 9018 | 185754 | 234992 | 357878 | 401190 |
| 4 | 9183 | 10078 | 187042 | 227245 | 301814 | 399389 |
| 5 | 8918 | 10399 | 208115 | 227818 | 383858 | 399203 |
| Mean | 8938.6 | 10069.2 | 194989.0 | 233760.2 | 359638.8 | 400071.0 |
| S.D (±) | 638.01 | 737.33 | 10601.62 | 6007.47 | 34316.15 | 1735.41 |
| C.V (%) | 7.14 | 7.32 | 5.44 | 2.57 | 9.54 | 0.43 |
| N | 5 | | 5 | | 5 | |
| % Recovery | 88.77 | | 83.41 | | 89.89 | |
| | | | | | | |
| | Overall Recovery of Ondansetron | | | | | |
| | | | | | | |
| | Mean | 87.36 | | | | |
| | S.D (±) | 3.4629 | | | | |
| | C.V (%) | 3.96 | | | | |
| | N | 3 | | | | |

**Table 14 b Recovery study of Etoricoxib (Internal Standard)**

| | Internal Standard Response | |
|---|---|---|
| | Extracted | Unextracted |
| 1 | 162766 | 157601 |
| 2 | 182341 | 146633 |
| 3 | 183395 | 149473 |
| 4 | 172782 | 150218 |
| 5 | 18846 | 151381 |
| Mean | 144026.0 | 151061.2 |
| S.D | 70473.55 | 4053.20 |
| C.V (%) | 48.93 | 2.68 |
| % Recovery | 95.34 | |

**Figure 22a Typical chromatogram of standard solution of Ondansetron**

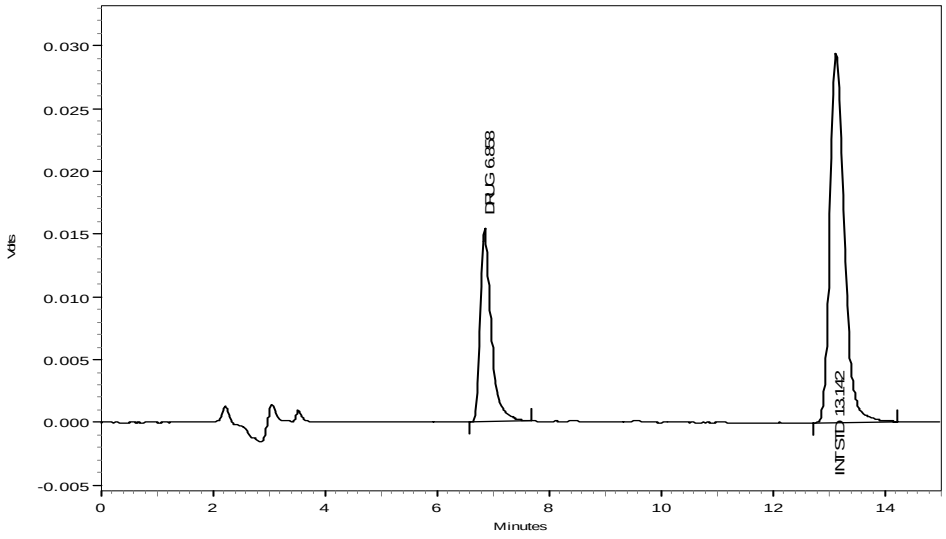



**Figure 22b Typical chromatogram of blank human plasma**

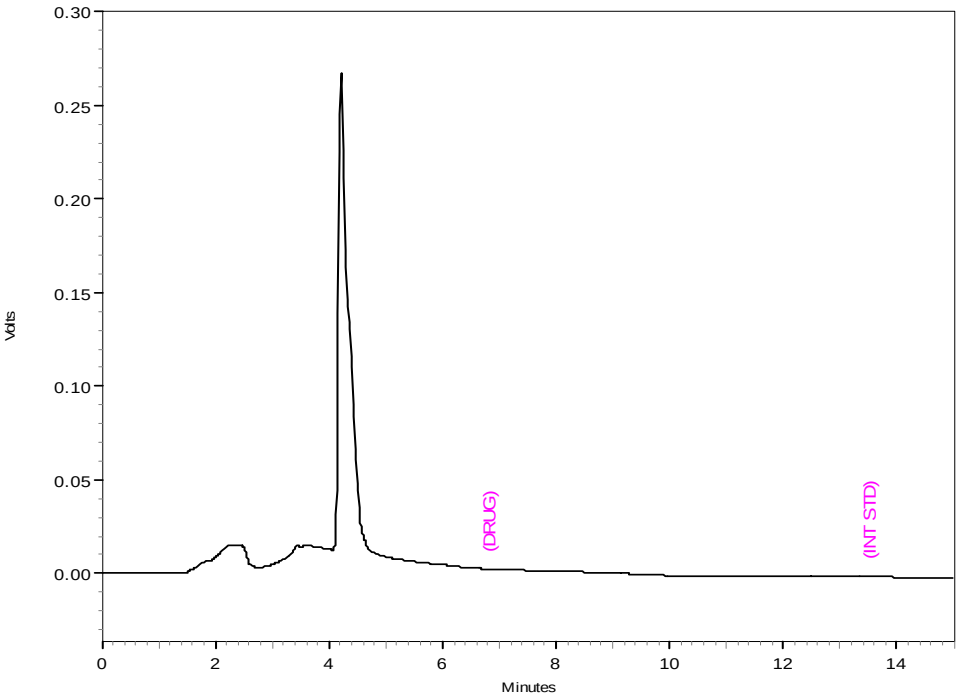


**Figure 22c Typical chromatogram of spiked human plasma**

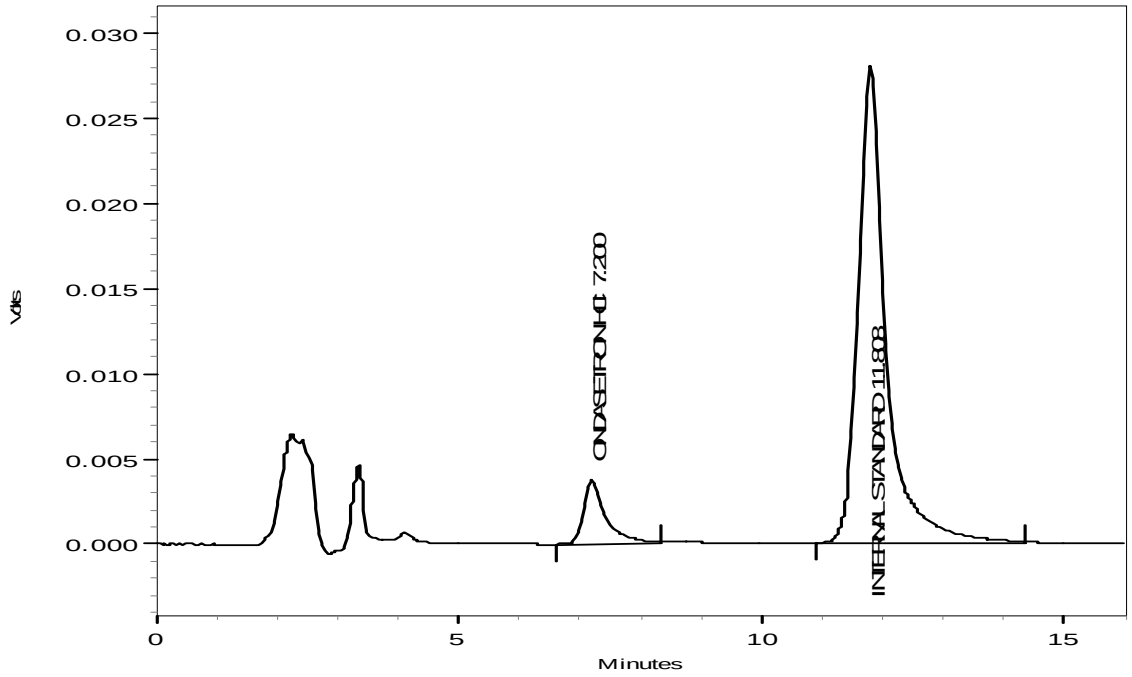



**Figure 22d Typical chromatogram of Ondansetron in human plasma at 1 h after a single oral dosage of 8 mg Ondansetron from a health volunteer**

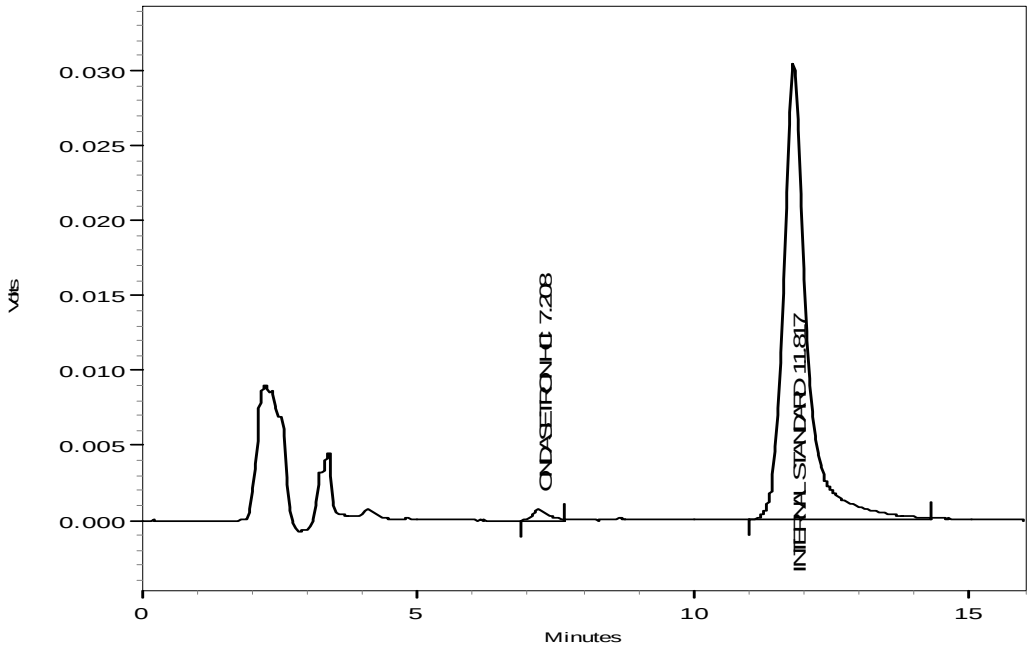


**Figure 22e Typical chromatogram of Ondansetron in human plasma at 4 h after a single oral dosage of 8 mg Ondansetron from a health volunteer**

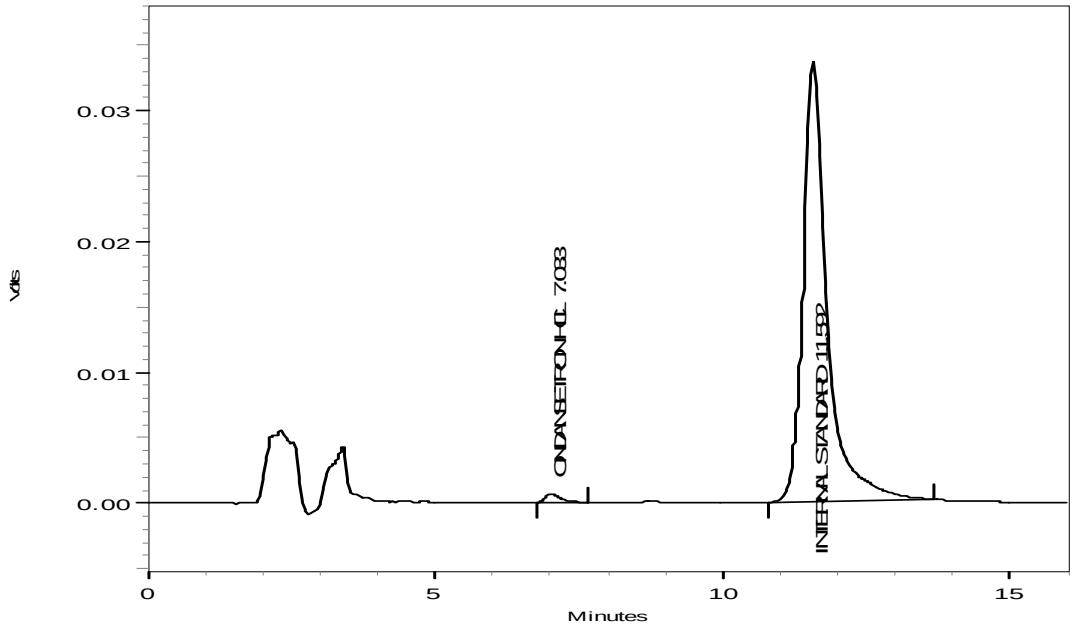



**Figure 22f Typical chromatogram of Ondansetron in human plasma at 12 h after a single oral dosage of 8 mg Ondansetron from a health volunteer**

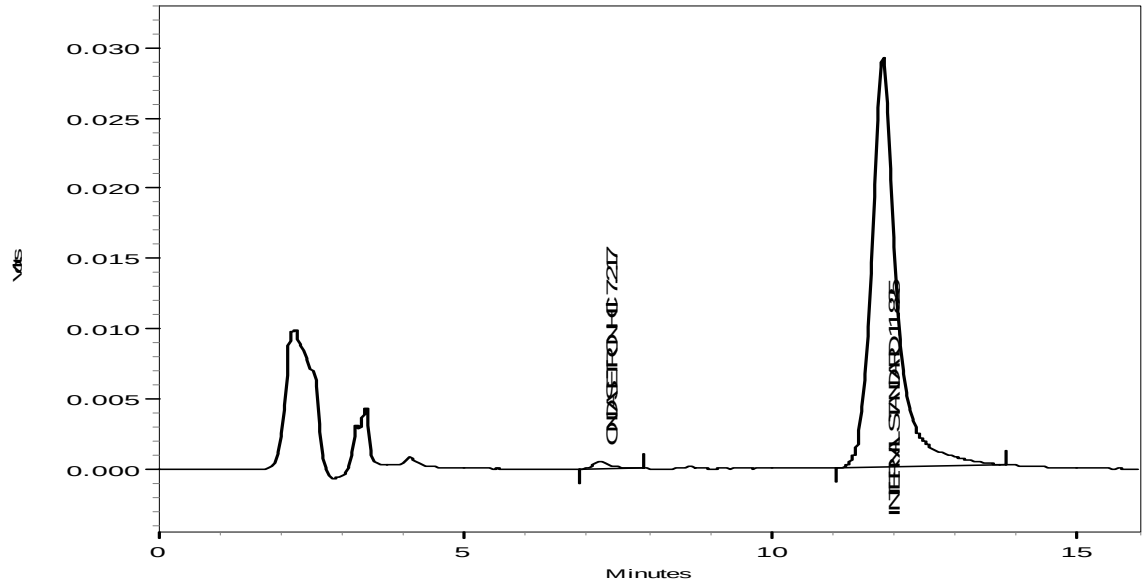


**Figure 23 Calibration curve of Ondansetron hydrochloride**

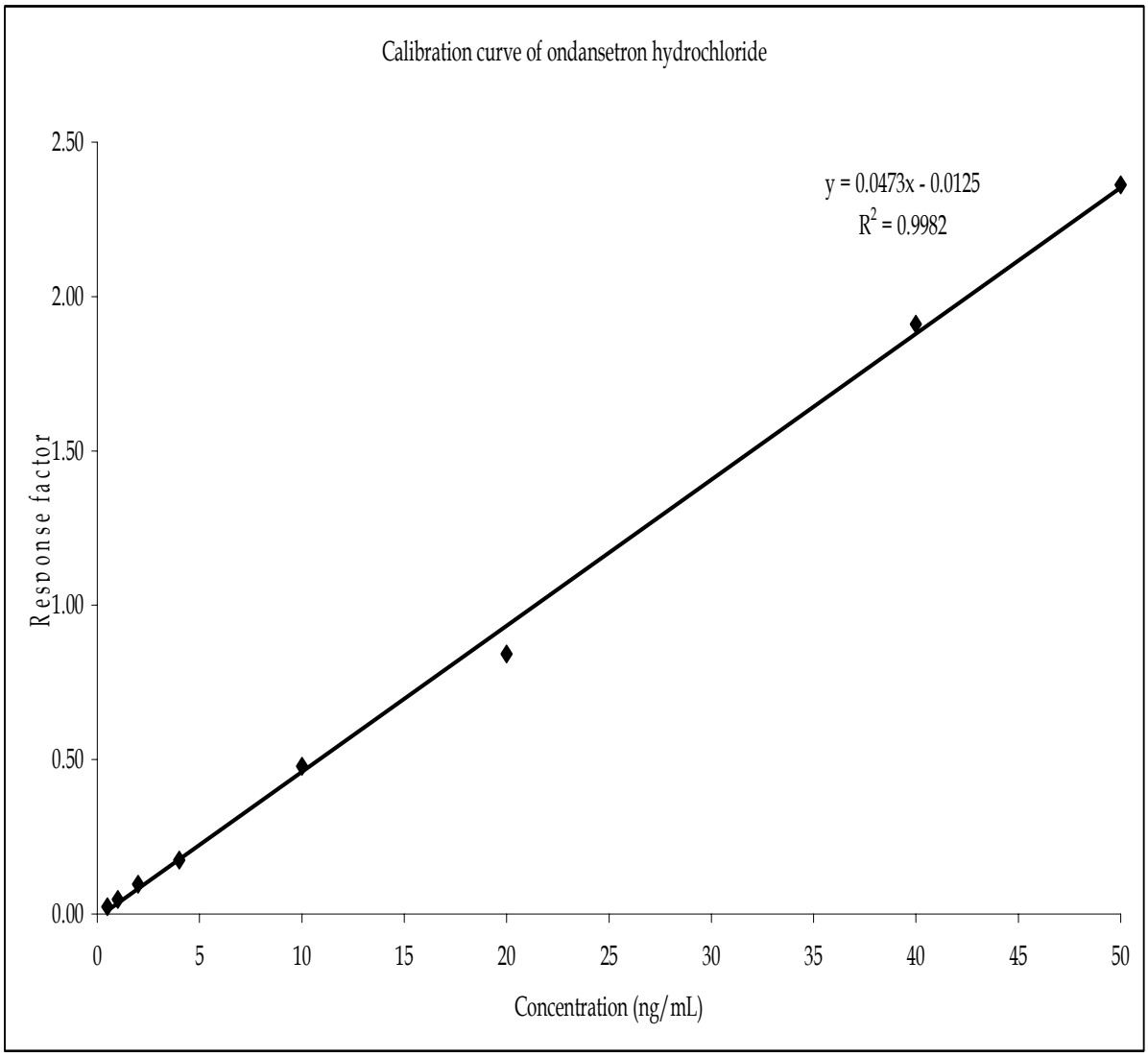


**Table 15 Concentrations-response Linearity Data for Dextromethorphan**

| Nominal Concentration (ng/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **110.0** | **140.0** | **230.0** | **590.0** | **1000.0** | **1400.0** | **2100.0** | **3000.0** |
| 1 | 109.635 | 139.115 | 231.045 | 564.382 | 897.624 | 1345.125 | 2145.679 | 2647.005 |
| 2 | 108.620 | 140.562 | 228.334 | 601.250 | 899.643 | 1564.465 | 2216.489 | 2976.324 |
| 3 | 110.061 | 136.954 | 236.431 | 611.207 | 910.784 | 1502.379 | 2343.000 | 2897.255 |
| 4 | 111.230 | 137.015 | 235.320 | 597.844 | 963.455 | 1498.954 | 2148.904 | 2986.788 |
| Mean | 109.8865 | 138.4115 | 232.7825 | 593.6708 | 917.8765 | 1477.7308 | 2213.5180 | 2876.8430 |
| S.D (±) | 1.08054 | 1.75063 | 3.76645 | 20.33234 | 30.93179 | 93.39000 | 92.28852 | 158.35248 |
| C.V (%) | 0.98 | 1.26 | 1.62 | 3.42 | 3.37 | 6.32 | 4.17 | 5.50 |
| % Nominal | 99.90 | 98.87 | 101.21 | 100.62 | 91.79 | 105.55 | 105.41 | 95.89 |
| N | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

## Table 16 a Within Batch Precision and Accuracy for Dextromethorphan

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | LLOQ QC | LQC | MQC | HQC |
| | **110.000** | **590.000** | **1400.000** | **3000.000** |
| 1 | 98.845 | 598.335 | 1346.288 | 2987.644 |
| 2 | 99.164 | 599.212 | 1362.555 | 2983.446 |
| 3 | 100.527 | 600.458 | 1398.472 | 2784.135 |
| 4 | 107.895 | 608.972 | 1496.557 | 2647.317 |
| 5 | 105.360 | 639.942 | 1322.080 | 2794.613 |
| Mean | 102.3582 | 609.3838 | 1385.1904 | 2839.4310 |
| S.D (±) | 4.04863 | 17.60103 | 68.16818 | 145.50043 |
| C.V (%) | 3.96 | 2.89 | 4.92 | 5.12 |
| % Nominal | 93.05 | 103.29 | 98.94 | 94.65 |
| N | 5 | 5 | 5 | 5 |
| 6 | 100.584 | 576.841 | 1384.950 | 2976.003 |
| 7 | 107.458 | 564.213 | 1399.416 | 3001.479 |
| 8 | 109.324 | 567.987 | 1400.574 | 2985.345 |
| 9 | 110.582 | 541.804 | 1397.332 | 2863.910 |
| 10 | 96.790 | 587.969 | 1399.176 | 3012.891 |
| Mean | 104.9476 | 567.7628 | 1396.2896 | 2967.9256 |
| S.D (±) | 5.97472 | 17.15528 | 6.44467 | 59.86577 |
| C.V (%) | 5.69 | 3.02 | 0.46 | 2.02 |
| % Nominal | 95.41 | 96.23 | 99.73 | 98.93 |
| N | 5 | 5 | 5 | 5 |
| 11 | 98.354 | 598.428 | 1346.945 | 2996.370 |
| 12 | 109.456 | 597.643 | 1394.670 | 3012.617 |
| 13 | 110.678 | 603.456 | 1404.612 | 2974.561 |
| 14 | 100.216 | 599.137 | 1397.104 | 2998.111 |
| 15 | 108.779 | 590.720 | 1399.337 | 3000.589 |
| Mean | 105.4966 | 597.8768 | 1388.5336 | 2996.4496 |
| S.D (±) | 5.74890 | 4.59058 | 23.53692 | 13.78765 |
| C.V (%) | 5.45 | 0.77 | 1.70 | 0.46 |
| % Nominal | 95.91 | 101.34 | 99.18 | 99.88 |
| N | 5 | 5 | 5 | 5 |
| 16 | 98.637 | 611.478 | 1399.770 | 2987.641 |
| 17 | 106.612 | 600.876 | 1432.589 | 2897.324 |
| 18 | 100.478 | 606.480 | 1428.952 | 3045.975 |
| 19 | 99.371 | 599.741 | 1419.076 | 3004.567 |
| 20 | 100.987 | 606.005 | 1401.657 | 2987.927 |
| Mean | 101.2170 | 604.9160 | 1416.4088 | 2984.6868 |
| S.D (±) | 3.15314 | 4.73799 | 15.17160 | 54.32069 |
| C.V (%) | 3.12 | 0.78 | 1.07 | 1.82 |
| % Nominal | 92.02 | 102.53 | 101.17 | 99.49 |
| N | 5 | 5 | 5 | 5 |

**Table 16 b Between Batch / Inter Day Precision and Accuracy for Dextromethorphan**

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | **110.000** | **590.000** | **1400.000** | **3000.000** |
| 1 | 98.845 | 598.335 | 1346.288 | 2987.644 |
| 2 | 99.164 | 599.212 | 1362.555 | 2983.446 |
| 3 | 100.527 | 600.458 | 1398.472 | 2784.135 |
| 4 | 107.895 | 608.972 | 1496.557 | 2647.317 |
| 5 | 105.360 | 639.942 | 1322.080 | 2794.613 |
| 6 | 100.584 | 576.841 | 1384.950 | 2976.003 |
| 7 | 107.458 | 564.213 | 1399.416 | 3001.479 |
| 8 | 109.324 | 567.987 | 1400.574 | 2985.345 |
| 9 | 110.582 | 541.804 | 1397.332 | 2863.910 |
| 10 | 96.790 | 587.969 | 1399.176 | 3012.891 |
| 11 | 98.354 | 598.428 | 1346.945 | 2996.370 |
| 12 | 109.456 | 597.643 | 1394.670 | 3012.617 |
| 13 | 110.678 | 603.456 | 1404.612 | 2974.561 |
| 14 | 100.216 | 599.137 | 1397.104 | 2998.111 |
| 15 | 108.779 | 590.720 | 1399.337 | 3000.589 |
| 16 | 98.637 | 611.478 | 1399.770 | 2987.641 |
| 17 | 106.612 | 600.876 | 1432.589 | 2897.324 |
| 18 | 100.478 | 606.480 | 1428.952 | 3045.975 |
| 19 | 99.371 | 599.741 | 1419.076 | 3004.567 |
| 20 | 100.987 | 606.005 | 1401.657 | 2987.927 |
| Mean | 103.5049 | 594.9849 | 1396.6056 | 2947.1233 |
| S.D (±) | 4.83034 | 20.34866 | 36.14958 | 100.25043 |
| C.V (%) | 4.67 | 3.42 | 2.59 | 3.40 |
| % Nominal | 94.10 | 100.84 | 99.76 | 98.24 |
| N | 20 | 20 | 20 | 20 |

**Table 16 c Intra Day Precision and Accuracy for Dextromethorphan**

| | Nominal Concentration (ng/ml) | | | |
|---|---|---|---|---|
| | LLOQ QC | LQC | MQC | HQC |
| | **110.000** | **590.000** | **1400.000** | **3000.000** |
| 1 | 98.845 | 598.335 | 1346.288 | 2987.644 |
| 2 | 99.164 | 599.212 | 1362.555 | 2983.446 |
| 3 | 100.527 | 600.458 | 1398.472 | 2784.135 |
| 4 | 107.895 | 608.972 | 1496.557 | 2647.317 |
| 5 | 105.360 | 639.942 | 1322.080 | 2794.613 |
| 6 | 100.584 | 576.841 | 1384.950 | 2976.003 |
| 7 | 107.458 | 564.213 | 1399.416 | 3001.479 |
| 8 | 109.324 | 567.987 | 1400.574 | 2985.345 |
| 9 | 110.582 | 541.804 | 1397.332 | 2863.910 |
| 10 | 96.790 | 587.969 | 1399.176 | 3012.891 |
| Mean | 103.6529 | 588.5733 | 1390.7400 | 2903.6783 |
| S.D (±) | 5.00130 | 27.38038 | 46.02139 | 124.85294 |
| C.V (%) | 4.83 | 4.65 | 3.31 | 4.30 |
| % Nominal | 94.23 | 99.76 | 99.34 | 96.79 |
| N | 10 | 10 | 10 | 10 |
| | Nominal Concentration (ng/ml) | | | |
| | LLOQ QC | LQC | MQC | HQC |
| | **110.000** | **590.000** | **1400.000** | **3000.000** |
| 11 | 98.354 | 598.428 | 1346.945 | 2996.370 |
| 12 | 109.456 | 597.643 | 1394.670 | 3012.617 |
| 13 | 110.678 | 603.456 | 1404.612 | 2974.561 |
| 14 | 100.216 | 599.137 | 1397.104 | 2998.111 |
| 15 | 108.779 | 590.720 | 1399.337 | 3000.589 |
| 16 | 98.637 | 611.478 | 1399.770 | 2987.641 |
| 17 | 106.612 | 600.876 | 1432.589 | 2897.324 |
| 18 | 100.478 | 606.480 | 1428.952 | 3045.975 |
| 19 | 99.371 | 599.741 | 1419.076 | 3004.567 |
| 20 | 100.987 | 606.005 | 1401.657 | 2987.927 |
| Mean | 103.3568 | 601.3964 | 1402.4712 | 2990.5682 |
| S.D (±) | 4.91885 | 5.75388 | 23.75623 | 37.87297 |
| C.V (%) | 4.76 | 0.96 | 1.69 | 1.27 |
| % Nominal | 93.96 | 101.93 | 100.18 | 99.69 |
| N | 10 | 10 | 10 | 10 |

**Table 17 a Stock Stability of Dextromethorphan**

| S.No | Drug area | Drug area | Drug area |
|---|---|---|---|
| | 0 h | 3 h | 6 h |
| 1 | 983464 | 987946 | 997645 |
| 2 | 978674 | 976455 | 978324 |
| 3 | 987849 | 964876 | 996407 |
| 4 | 987645 | 987456 | 983710 |
| 5 | 990146 | 987669 | 984675 |
| Mean | 985555.6 | 980880.4 | 988152.2 |
| S.D | 4539.50 | 10185.42 | 8465.70 |
| CV(%) | 0.46 | 1.04 | 0.86 |

**Table 17 b -20°C Stability of Dextromethorphan in plasma**

| | Nominal Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **590.000** | **3000.000** |
| | 576.481 | 2976.481 |
| | 591.748 | 2983.614 |
| | 580.125 | 2948.325 |
| | 593.546 | 2690.401 |
| | 590.451 | 2956.234 |
| Mean | 586.4702 | 2911.0110 |
| S.D. | 7.64550 | 124.16093 |
| CV(%) | 1.30 | 4.27 |
| % Nominal | 99.40 | 97.03 |
| N | 5 | 5 |

**Table 17 c Short Term Room Temperature Stability of Dextromethorphan**

| | Nominal Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **590.000** | **3000.000** |
| | 596.348 | 2974.784 |
| | 590.001 | 3015.434 |
| | 498.762 | 2935.628 |
| | 499.325 | 2673.354 |
| | 503.789 | 2876.925 |
| Mean | 537.6450 | 2895.2250 |
| S.D | 50.77827 | 134.13973 |
| C.V(%) | 9.44 | 4.63 |
| % Nominal | 91.13 | 96.51 |
| N | 5 | 5 |

**Table 17 d Auto sampler Stability of Dextromethorphan in plasma**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **590.000** | **3000.000** |
| | 593.412 | 2674.156 |
| | 590.121 | 2845.671 |
| | 587.040 | 2946.013 |
| | 576.374 | 2846.659 |
| | 580.638 | 2901.389 |
| Mean | 585.5170 | 2842.7776 |
| S.D | 6.95066 | 103.13768 |
| CV (%) | 1.19 | 3.63 |
| % Nominal | 99.24 | 94.76 |
| N | 5 | 5 |

**Table 17 e Freeze/thaw cycle stability**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **590.000** | **3000.000** |
| | 587.643 | 2634.146 |
| | 590.357 | 2789.180 |
| | 592.887 | 2894.115 |
| | 593.317 | 2930.157 |
| | 590.478 | 3001.489 |
| Mean | 590.9364 | 2849.8174 |
| S.D (±) | 2.28388 | 142.83911 |
| C.V (%) | 0.39 | 5.01 |
| % Nominal | 100.16 | 94.99 |
| N | 5 | 5 |

**Table 17 f Long term stability for four weeks**

| | Concentration (ng/ml) | |
|---|---|---|
| | LQC | HQC |
| | **590.000** | **3000.000** |
| | 586.134 | 2987.654 |
| | 551.470 | 2998.765 |
| | 580.846 | 3011.823 |
| | 576.481 | 2634.211 |
| | 563.953 | 2746.623 |
| Mean | 571.7768 | 2875.8152 |
| S.D (±) | 14.00078 | 174.05883 |
| C.V (%) | 2.45 | 6.05 |
| % Nominal | 96.91 | 95.86 |
| N | 5 | 5 |

**Table 18 a Recovery study of Dextromethorphan**

| | LQC Response | | MQC Response | | HQC Response | |
|---|---|---|---|---|---|---|
| | Extracted | Unextracted | Extracted | Unextracted | Extracted | Unextracted |
| 1 | 16542 | 17264 | 534261 | 632472 | 846799 | 965788 |
| 2 | 16378 | 17647 | 613483 | 534385 | 869586 | 942356 |
| 3 | 16549 | 16943 | 593460 | 615126 | 896301 | 901467 |
| 4 | 16634 | 16832 | 576324 | 654301 | 864535 | 879524 |
| 5 | 16349 | 16139 | 584315 | 554895 | 901245 | 864731 |
| Mean | 16490.4 | 16965 | 591895.5 | 598235.8 | 875693.2 | 910773.2 |
| S.D | 121.80 | 560.13 | 16004.20 | 51371.33 | 22771.99 | 42447.33 |
| C.V | 0.74 | 3.30 | 2.70 | 8.59 | 2.60 | 4.66 |
| n | 5 | | 5 | | 5 | |
| % Recovery | 97.20 | | 98.94 | | 96.15 | |
| | Overall Recovery of Dextromethorphan | | | | | |
| | | | | | | |
| | Mean | 97.43 | | | | |
| | S.D (±) | 1.4098 | | | | |
| | C.V (%) | 1.45 | | | | |
| | N | 3 | | | | |

**Table 18 b Recovery of Losartan potassium (Internal standard)**

| | Internal Standard Response | |
|---|---|---|
| | Extracted | Unextracted |
| 1 | 487999 | 634488 |
| 2 | 493468 | 563012 |
| 3 | 493012 | 637948 |
| 4 | 489586 | 582301 |
| 5 | 486251 | 542688 |
| Mean | 490063.2 | 592087.4 |
| S.D | 3134.87 | 42668.66 |
| C.V(%) | 0.64 | 7.21 |
| % Recovery | 82.769 | |

**Figure 24a Typical chromatogram of standard solution of Dextromethorphan**

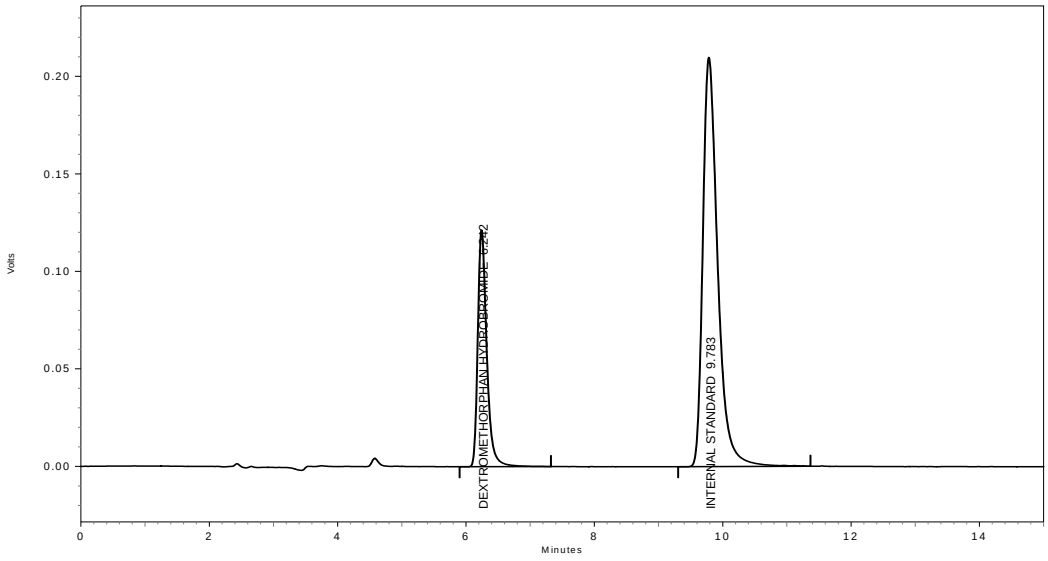



**Figure 24b Typical chromatogram of blank human plasma**

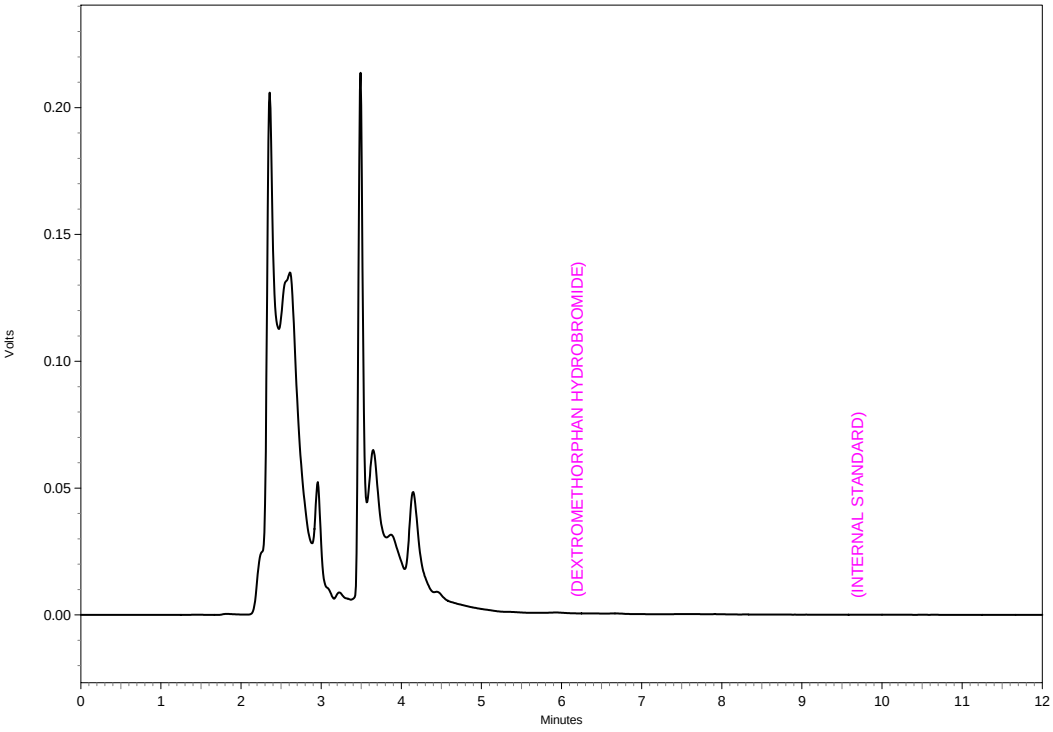


**Figure 24c Typical chromatogram of Dextromethorphan in human plasma at 1 h after a single oral dosage of 60mg Dextromethorphan from a health volunteer**

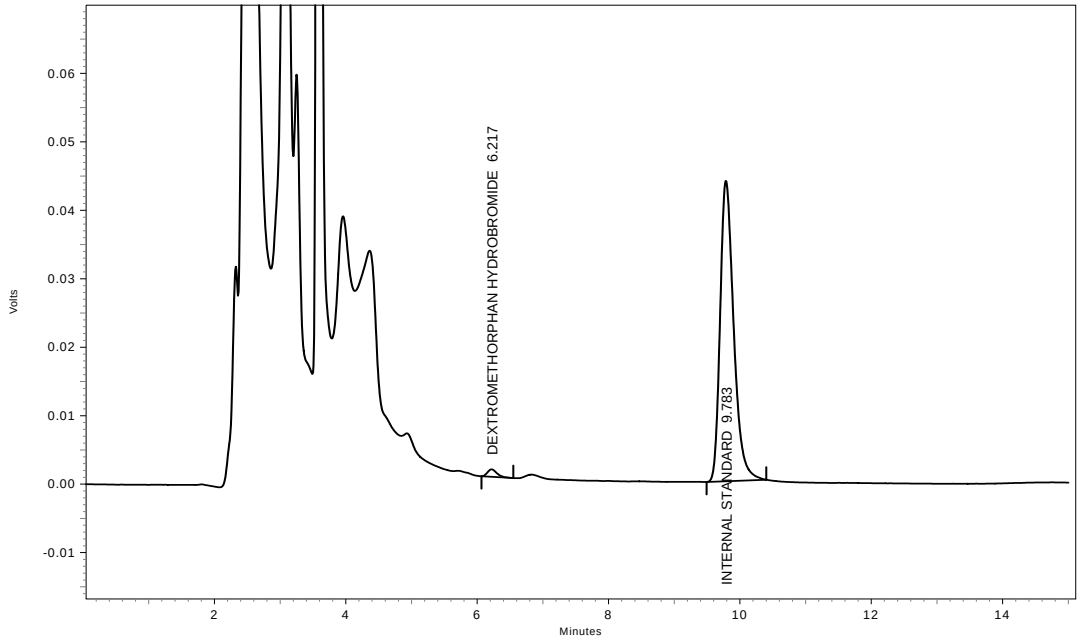



**Figure 24d Typical chromatogram of Dextromethorphan in human plasma at 4 h after a single oral dosage of 60mg Dextromethorphan from a health volunteer**

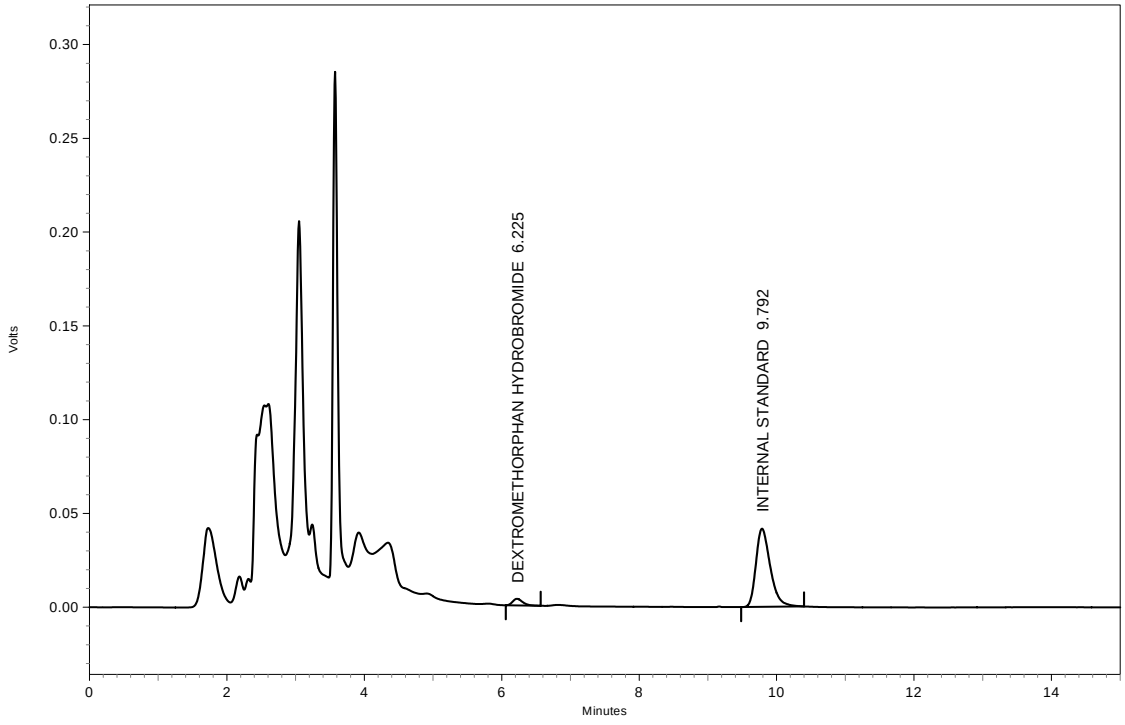


**Figure 25 Calibration curve of Dextromethorphan hydrobromide**

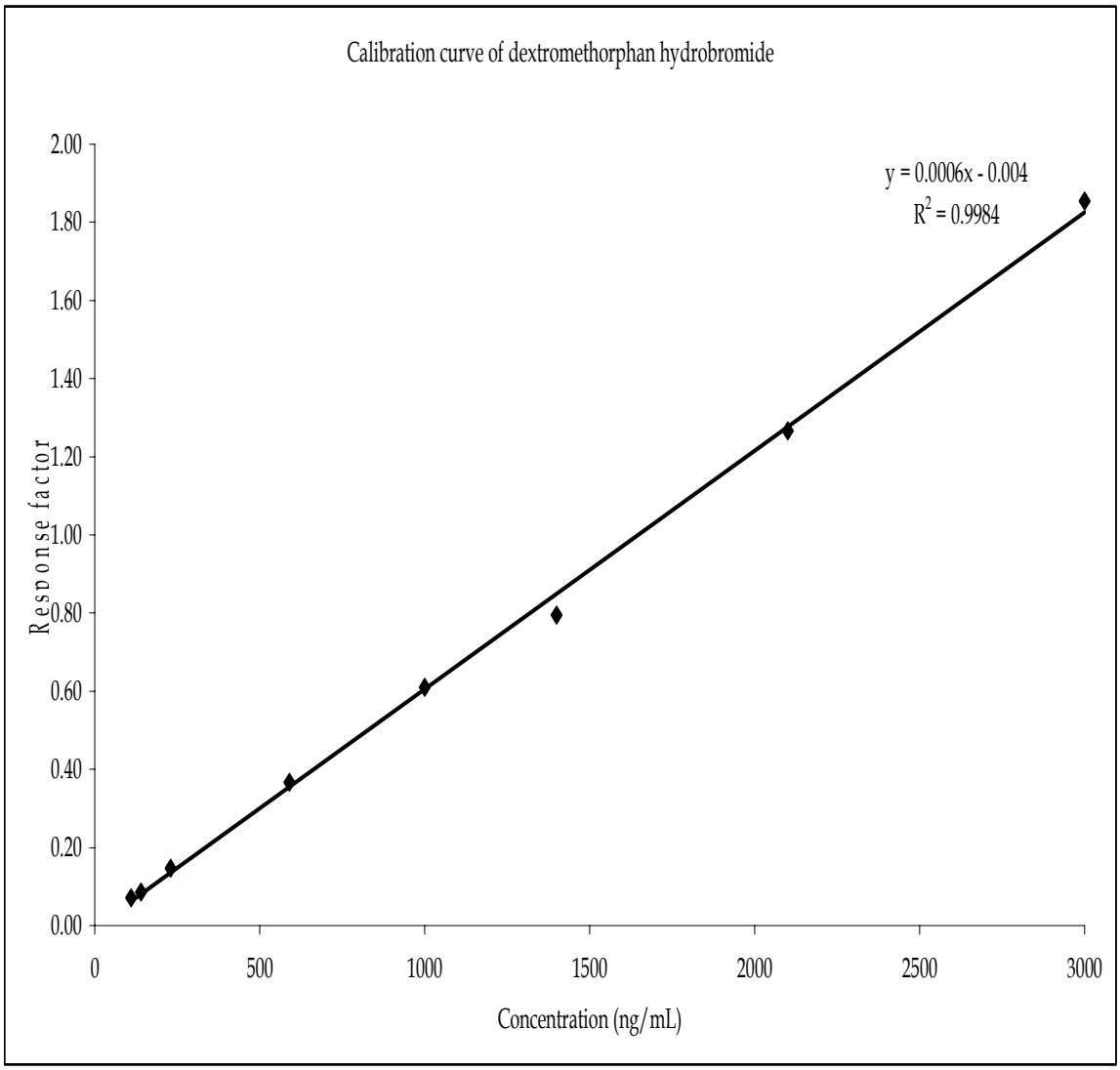


**Table 19 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Ondansetron immediate release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.50 | 5.369 | 4.988 | 1.994 | 3.308 | 9.573 | 7.615 |
| 1.00 | 20.078 | 18.238 | 5.165 | 11.549 | 21.631 | 13.4 |
| 1.50 | 41.516 | 34.427 | 12.012 | 22.962 | 35.835 | 31.875 |
| 2.00 | 35.765 | 39.117 | 20.47 | 41.038 | 43.628 | 40.693 |
| 2.50 | 24.405 | 34.542 | 36.579 | 36.342 | 37.519 | 36.931 |
| 3.00 | 20.337 | 20.117 | 26.282 | 21.825 | 34.448 | 28.961 |
| 4.00 | 13.332 | 12.554 | 22.532 | 16.355 | 25.68 | 19.825 |
| 6.00 | 8.05 | 9.238 | 19.81 | 11.544 | 12.301 | 12.883 |
| 8.00 | 5.208 | 4.847 | 12.69 | 7.858 | 8.114 | 7.209 |
| 12.00 | 2.694 | 1.669 | 4.958 | 3.648 | 3.251 | 3.861 |
| 18.00 | 1.327 | 1.033 | 1.197 | 0.864 | 2.69 | 2.017 |
| 24.00 | 0.986 | 0.976 | 0.733 | 0.8017 | 1.093 | 1.099 |
| **$C_{max}$** | **41.516** | **39.117** | **36.579** | **41.038** | **43.628** | **40.693** |
| **$T_{max}$** | **1.500** | **2.000** | **2.500** | **2.000** | **2.000** | **2.000** |
| **$AUC_{0-t}$** | **154.931** | **150.063** | **203.481** | **170.992** | **223.067** | **198.812** |
| **$t_{1/2}$** | **4.120** | **3.869** | **4.145** | **3.720** | **4.144** | **4.185** |
| **$K_{el}$** | **0.168** | **0.175** | **0.188** | **0.186** | **0.167** | **0.166** |
| **$AUC_{0-\infty}$** | **160.792** | **155.652** | **207.370** | **175.295** | **229.601** | **205.448** |

[a] Volunteers

**Table 20 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Ondansetron slow modified release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.50 | 4.807 | 8.835 | 2.831 | 3.877 | 4.313 | 5.075 |
| 1.00 | 9.375 | 12.462 | 7.519 | 6.886 | 13.516 | 11.01 |
| 1.50 | 12.83 | 15.38 | 12.977 | 12.706 | 24.527 | 15.837 |
| 2.00 | 15.872 | 20.051 | 16.699 | 17.215 | 34.467 | 26.923 |
| 2.50 | 20.479 | 27.617 | 20.898 | 23.99 | 39.312 | 33.646 |
| 3.00 | 28.086 | 34.379 | 33.439 | 31.449 | 43.241 | 42.379 |
| 4.00 | 43.332 | 43.079 | 36.555 | 36.327 | 44.213 | 44.884 |
| 6.00 | 45.73 | 47.786 | 28.943 | 45.948 | 40.411 | 48.163 |
| 8.00 | 30.952 | 30.115 | 22.545 | 31.825 | 30.861 | 39.08 |
| 12.00 | 23.764 | 15.076 | 16.333 | 18.032 | 21.148 | 23.64 |
| 18.00 | 7.632 | 11.684 | 10.631 | 8.957 | 6.557 | 7.82 |
| 24.00 | 2.208 | 1.961 | 2.107 | 1.527 | 1.58 | 1.351 |
| **$C_{max}$** | **45.730** | **47.786** | **36.555** | **45.948** | **44.213** | **48.163** |
| **$T_{max}$** | **6.000** | **6.000** | **4.000** | **6.000** | **4.000** | **6.000** |
| **$AUC_{0-t}$** | **473.296** | **469.859** | **387.667** | **446.268** | **480.045** | **528.095** |
| **$t_{1/2}$** | **4.201** | **4.344** | **5.354** | **3.891** | **4.149** | **3.545** |
| **$K_{el}$** | **0.165** | **0.160** | **0.129** | **0.178** | **0.167** | **0.196** |
| **$AUC_{0-\infty}$** | **486.679** | **482.149** | **403.943** | **454.841** | **489.502** | **535.005** |

[a] Volunteers

**Table 21 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Ondansetron fast modified release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.50 | 5.359 | 7.225 | 2.164 | 3.576 | 4.635 | 7.579 |
| 1.00 | 15.748 | 19.268 | 9.615 | 19.154 | 13.924 | 16.447 |
| 1.50 | 23.601 | 27.016 | 12.474 | 24.466 | 23.761 | 27.643 |
| 2.00 | 34.526 | 38.102 | 26.65 | 27.025 | 30.522 | 29.107 |
| 2.50 | 39.152 | 42.287 | 30.561 | 31.924 | 36.12 | 33.004 |
| 3.00 | 40.239 | 45.632 | 36.228 | 38.915 | 39.233 | 40.895 |
| 4.00 | 42.912 | 47.926 | 46.451 | 40.103 | 43.021 | 42.321 |
| 6.00 | 36.824 | 33.693 | 34.674 | 46.678 | 43.002 | 42.965 |
| 8.00 | 28.611 | 16.425 | 28.711 | 35.923 | 31.554 | 29.864 |
| 12.00 | 10.893 | 8.394 | 15.898 | 13.784 | 16.312 | 11.756 |
| 18.00 | 6.342 | 4.728 | 13.679 | 11.015 | 13.667 | 8.997 |
| 24.00 | 1.944 | 2.607 | 2.029 | 1.841 | 2.238 | 2.677 |
| **$C_{max}$** | **42.912** | **47.926** | **46.451** | **46.678** | **43.021** | **42.965** |
| **$T_{max}$** | **4.000** | **4.000** | **4.000** | **6.000** | **4.000** | **6.000** |
| **$AUC_{0-t}$** | **411.570** | **367.882** | **460.712** | **484.071** | **499.379** | **447.358** |
| **$t_{1/2}$** | **4.454** | **4.793** | **4.977** | **4.156** | **4.991** | **4.803** |
| **$K_{el}$** | **0.156** | **0.145** | **0.139** | **0.167** | **0.139** | **0.144** |
| **$AUC_{0-\infty}$** | **424.062** | **385.908** | **475.279** | **495.110** | **515.493** | **465.906** |

[a] Volunteers

**Table 22 Mean plasma concentrations (ng/ml) for Ondansetron hydrochloride**

| Time (h) | Immediate release formulation | | Slow MR[a] formulation | | Fast MR[a] formulation | |
|---|---|---|---|---|---|---|
| | Mean | S.D[b] | Mean | S.D[b] | Mean | S.D[b] |
| 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.50 | 5.475 | 2.773 | 4.956 | 2.058 | 5.090 | 2.092 |
| 1.00 | 15.010 | 6.188 | 10.128 | 2.668 | 15.693 | 3.617 |
| 1.50 | 29.771 | 10.607 | 15.710 | 4.531 | 23.160 | 5.503 |
| 2.00 | 36.785 | 8.401 | 21.871 | 7.374 | 30.989 | 4.503 |
| 2.50 | 34.386 | 4.991 | 27.657 | 7.515 | 35.508 | 4.543 |
| 3.00 | 25.328 | 5.685 | 35.496 | 6.068 | 40.190 | 3.110 |
| 4.00 | 18.380 | 5.218 | 41.398 | 3.894 | 43.789 | 2.874 |
| 6.00 | 12.304 | 4.117 | 42.830 | 7.346 | 39.639 | 5.289 |
| 8.00 | 7.654 | 2.817 | 30.896 | 5.257 | 28.515 | 6.514 |
| 12.00 | 3.347 | 1.114 | 19.666 | 3.732 | 12.840 | 3.067 |
| 18.00 | 1.521 | 0.696 | 8.880 | 1.950 | 9.738 | 3.736 |
| 24.00 | 0.948 | 0.151 | 1.789 | 0.349 | 2.223 | 0.351 |

[a] Modified Release
[b] Standard Deviation

**Table 23 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Dextromethorphan immediate release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.50 | 923.973 | 636.823 | 646.073 | 530.821 | 696.155 | 684.710 |
| 1.00 | 1053.257 | 944.748 | 1093.906 | 858.529 | 1126.013 | 1070.573 |
| 1.50 | 1148.006 | 1020.294 | 1305.807 | 1019.882 | 1244.090 | 1282.150 |
| 2.00 | 948.398 | 1183.262 | 1060.535 | 1214.336 | 1175.274 | 1111.618 |
| 2.50 | 818.526 | 1041.099 | 940.718 | 1007.181 | 892.198 | 922.701 |
| 3.00 | 757.591 | 935.885 | 726.525 | 1030.392 | 731.777 | 750.076 |
| 4.00 | 595.868 | 788.371 | 503.147 | 863.243 | 715.677 | 646.084 |
| 6.00 | 311.919 | 319.727 | 345.474 | 486.752 | 288.879 | 312.696 |
| 8.00 | 0.000 | 0.000 | 259.409 | 236.749 | 234.989 | 231.146 |
| 12.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 18.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 24.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **$C_{max}$** | **1148.006** | **1183.262** | **1305.807** | **1214.336** | **1244.090** | **1282.150** |
| **$T_{max}$** | **1.500** | **2.000** | **1.500** | **2.000** | **1.500** | **1.500** |
| **$AUC_{0-t}$** | **4531.912** | **4937.038** | **5292.308** | **6066.783** | **5471.937** | **5386.387** |
| **$t_{1/2}$** | **2.484** | **2.147** | **2.770** | **2.619** | **2.541** | **2.555** |
| **$K_{el}$** | **0.279** | **0.323** | **0.250** | **0.265** | **0.273** | **0.271** |
| **$AUC_{0-\infty}$** | **5649.665** | **5927.162** | **6328.971** | **6961.295** | **6333.249** | **6238.523** |

[a] Volunteers

**Table 24 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Dextromethorphan slow modified release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.50 | 580.593 | 600.635 | 706.103 | 460.435 | 490.653 | 703.305 |
| 1.00 | 809.825 | 937.750 | 1001.767 | 603.504 | 710.830 | 899.000 |
| 1.50 | 917.006 | 1267.778 | 1197.907 | 971.029 | 866.077 | 1377.539 |
| 2.00 | 1054.788 | 1732.780 | 1454.485 | 1361.316 | 1120.531 | 1730.402 |
| 2.50 | 1190.238 | 1977.670 | 1737.844 | 1517.864 | 1338.791 | 1903.825 |
| 3.00 | 1522.519 | 2095.945 | 1987.560 | 1453.042 | 1644.426 | 1913.531 |
| 4.00 | 1742.922 | 2202.944 | 2125.488 | 1840.852 | 1932.504 | 2115.185 |
| 6.00 | 1835.272 | 2245.000 | 2180.012 | 1865.245 | 2046.545 | 2200.661 |
| 8.00 | 1679.861 | 1661.634 | 1975.220 | 2013.424 | 1806.157 | 1407.147 |
| 12.00 | 941.693 | 593.410 | 665.201 | 873.501 | 801.960 | 825.136 |
| 18.00 | 693.124 | 572.984 | 684.550 | 631.976 | 738.218 | 843.001 |
| 24.00 | 186.315 | 285.145 | 197.673 | 169.272 | 167.000 | 132.974 |
| **$C_{max}$** | **1835.272** | **2245.000** | **2180.012** | **2013.424** | **2046.545** | **2200.661** |
| **$T_{max}$** | **6.000** | **6.000** | **6.000** | **8.000** | **6.000** | **6.000** |
| **$AUC_{0-t}$** | **24168.779** | **24869.969** | **26039.959** | **24746.068** | **24847.183** | **26120.334** |
| **$t_{1/2}$** | **5.731** | **6.423** | **5.503** | **4.847** | **5.359** | **5.180** |
| **$K_{el}$** | **0.121** | **0.108** | **0.126** | **0.143** | **0.129** | **0.134** |
| **$AUC_{0-\infty}$** | **39343.065** | **45673.620** | **41721.575** | **38826.086** | **38812.561** | **36635.220** |

[a] Volunteers

**Table 25 Individual plasma concentrations (ng/ml) and pharmacokinetic parameters for Dextromethorphan fast modified release product**

| Time | $V_1$[a] | $V_2$[a] | $V_3$[a] | $V_4$[a] | $V_5$[a] | $V_6$[a] |
|---|---|---|---|---|---|---|
| 0.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.50 | 506.830 | 633.128 | 612.373 | 576.669 | 674.190 | 678.814 |
| 1.00 | 832.732 | 992.324 | 844.965 | 657.999 | 819.037 | 937.528 |
| 1.50 | 1075.019 | 1185.641 | 1095.856 | 1100.166 | 1057.757 | 1136.837 |
| 2.00 | 1339.275 | 1714.178 | 1277.461 | 1517.849 | 1341.012 | 1178.997 |
| 2.50 | 1689.506 | 1913.590 | 1855.312 | 1820.882 | 1695.343 | 1293.912 |
| 3.00 | 1831.718 | 2110.007 | 1907.697 | 1885.700 | 1840.201 | 1772.383 |
| 4.00 | 1971.054 | 2290.825 | 2215.193 | 1982.085 | 2010.975 | 2239.062 |
| 6.00 | 2010.160 | 2323.468 | 2209.124 | 2234.008 | 2128.104 | 2147.051 |
| 8.00 | 1413.212 | 2109.022 | 2123.545 | 1789.232 | 1834.365 | 1949.937 |
| 12.00 | 1050.875 | 1482.551 | 1510.666 | 955.090 | 1404.027 | 1937.895 |
| 18.00 | 739.227 | 964.316 | 689.635 | 533.226 | 768.319 | 697.549 |
| 24.00 | 132.050 | 218.297 | 132.594 | 236.666 | 241.598 | 223.918 |
| **$C_{max}$** | **2010.160** | **2323.468** | **2215.193** | **2234.008** | **2128.104** | **2239.062** |
| **$T_{max}$** | **6.000** | **6.000** | **4.000** | **6.000** | **6.000** | **4.000** |
| **$AUC_{0-t}$** | **25397.894** | **33065.715** | **30474.351** | **25744.699** | **29304.424** | **31991.358** |
| **$t_{1/2}$** | **5.114** | **5.585** | **4.529** | **5.653** | **5.932** | **5.475** |
| **$K_{el}$** | **0.136** | **0.124** | **0.153** | **0.123** | **0.117** | **0.127** |
| **$AUC_{0-\infty}$** | **40229.987** | **51787.538** | **44349.049** | **40337.291** | **45003.298** | **47392.153** |

[a] Volunteers

**Table 26 Mean plasma concentrations (ng/ml) for Dextromethorphan hydrobromide**

| Time (h) | Immediate release formulation | | Slow MR[a] formulation | | Fast MR[a] formulation | |
|---|---|---|---|---|---|---|
| | Mean | S.D[b] | Mean | S.D[b] | Mean | S.D[b] |
| 0.00 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.50 | 686.426 | 130.252 | 590.287 | 103.127 | 613.667 | 64.916 |
| 1.00 | 1024.504 | 101.959 | 827.113 | 149.488 | 847.431 | 114.912 |
| 1.50 | 1170.038 | 128.017 | 1099.556 | 209.572 | 1108.546 | 46.214 |
| 2.00 | 1115.571 | 98.886 | 1409.050 | 290.226 | 1394.795 | 191.551 |
| 2.50 | 937.071 | 80.030 | 1611.039 | 314.991 | 1711.424 | 222.985 |
| 3.00 | 822.041 | 128.821 | 1769.504 | 265.198 | 1891.284 | 116.992 |
| 4.00 | 685.398 | 131.092 | 1993.316 | 182.157 | 2118.199 | 145.252 |
| 6.00 | 344.241 | 72.134 | 2062.122 | 177.200 | 2175.319 | 106.622 |
| 8.00 | 160.382 | 124.625 | 1757.240 | 225.047 | 1869.885 | 262.395 |
| 12.00 | 0.000 | 0.000 | 783.483 | 130.636 | 1390.184 | 354.306 |
| 18.00 | 0.000 | 0.000 | 693.976 | 92.454 | 732.045 | 139.882 |
| 24.00 | 0.000 | 0.000 | 189.730 | 51.643 | 197.520 | 51.196 |

[a] Modified Release
[b] Standard Deviation

**Table 27 Mean pharmacokinetic profile (n=6)**

| Drug name | | $C_{max}$[d] | $T_{max}$[d] | $AUC_{0-t}$[d] | $t_{1/2}$[d] | $k_{el}$[d] | $AUC_{0-\infty}$[d] |
|---|---|---|---|---|---|---|---|
| Ondansetron hydrochloride | IR[a] | 40.429 (2.383) | 2.000 (0.316) | 184.162 (29.951) | 4.030 (0.190) | 0.175 (0.010) | 188.973 (29.502) |
| | SMRT[b] | 44.732 (4.259) | 5.333 (1.033) | 464.205 (46.103) | 4.248 (0.611) | 0.166 (0.022) | 475.353 (43.481) |
| | FMRT[c] | 44.992 (2.276) | 4.667 (1.033) | 445.162 (48.581) | 4.695 (0.328) | 0.148 (0.011) | 460.293 (47.668) |
| Dextromethorphan Hydrobromide | IR[a] | 1229.608 (59.694) | 1.667 (0.258) | 5281.061 (518.573) | 2.519 (0.207) | 0.277 (0.025) | 6239.811 (443.451) |
| | SMRT[b] | 2086.819 (152.939) | 6.333 (0.816) | 25132.049 (778.166) | 5.507 (0.539) | 0.127 (0.012) | 40168.688 (3147.203) |
| | FMRT[c] | 2191.666 (108.581) | 5.333 (1.033) | 29329.740 (3183.219) | 5.381 (0.495) | 0.130 (0.013) | 44849.886 (4394.895) |

[a] Immediate Release

[b] Slow Modified Release Tablets

[c] Fast Modified Release Tablets

[d] Results represents the mean of replicate determination with the standard deviation given in parenthesis

**Figure 26 Mean plasma concentration-time profile of Ondansetron from developed Sustained release tablets (test) and marketed immediate release tablet (Reference)**

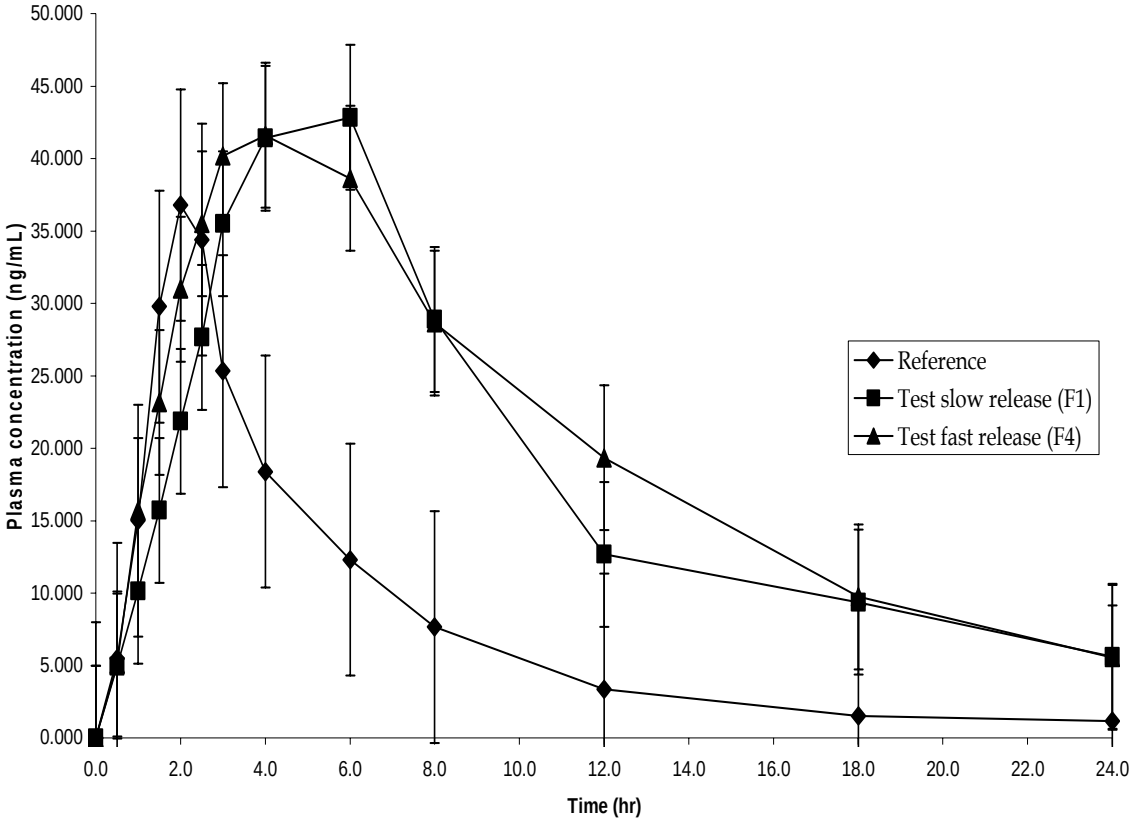
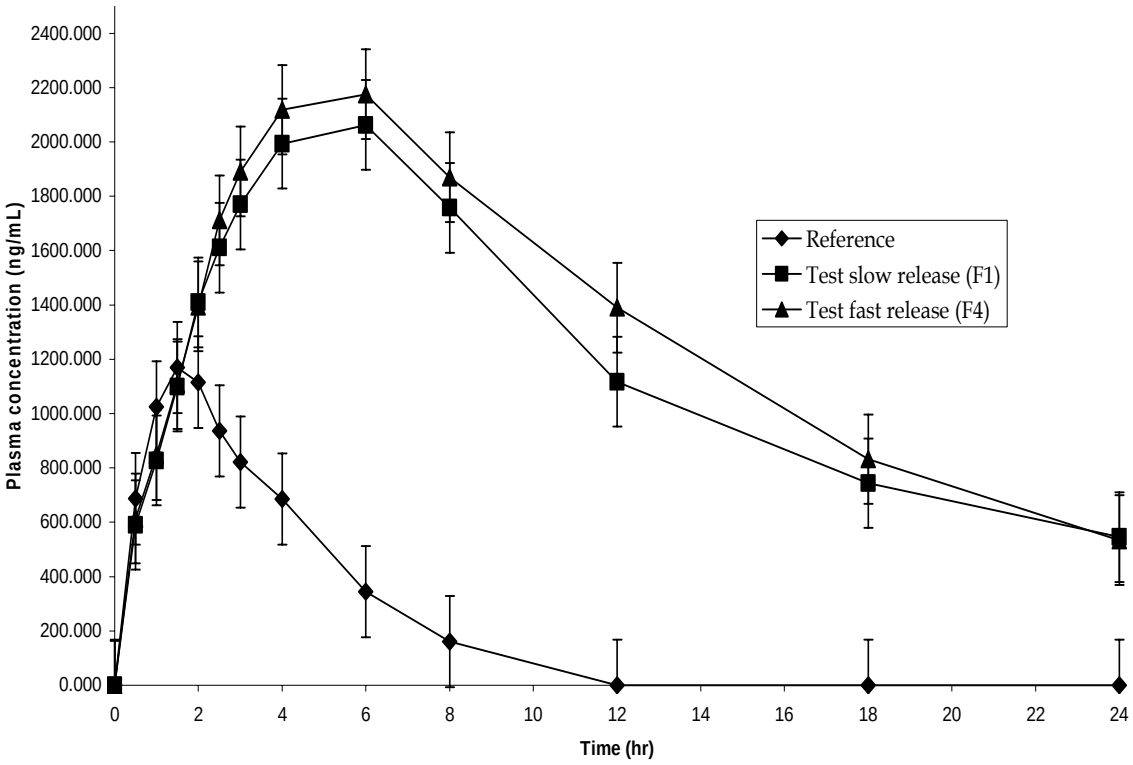


**Figure 27 Mean plasma concentration-time profile of Dextromethorphan from developed Sustained release tablets (test) and marketed immediate release tablet (Reference)**



## Table 28
## Statistical data for Ondansetron hydrochloride slow versus reference release formulations

Dependent Variable: $C_{max}$

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 9.112 | 1 | 9.112 | 6.002E3 | .000 | .999 | 6002.359 | 1.000 |
| | Error | .012 | 7.906 | .002[b] | | | | | |
| Period | Hypothesis | .028 | 1 | .028 | 27.802 | .006 | .874 | 27.802 | .970 |
| | Error | .004 | 4 | .001[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .053 | 5 | .011 | 10.605 | .020 | .930 | 53.025 | .855 |
| | Error | .004 | 4 | .001[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 29
## Paired Samples Test

| | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | Lower | Upper | | | |
| Pair 1 $C_{max}$slow - $C_{max}$ref | .09667 | .08017 | .03273 | .01254 | .18080 | 2.954 | 5 | .032 |

## Table 30 Tests of Between-Subjects Effects

Dependent Variable:auc0tot

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 30.317 | 1 | 30.317 | 1.685E3 | .000 | .997 | 1685.489 | 1.000 |
| | Error | .080 | 4.424 | .018[b] | | | | | |
| Period | Hypothesis | 2.613 | 1 | 2.613 | 144.917 | .000 | .973 | 144.917 | 1.000 |
| | Error | .072 | 4 | .018[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .086 | 5 | .017 | .951 | .534 | .543 | 4.756 | .139 |
| | Error | .072 | 4 | .018[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 31 Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0totslow - auc0totref | .93333 | .19826 | .08094 | .72527 | 1.14139 | 11.531 | 5 | .000 |

## Table 32 Tests of Between-Subjects Effects

Dependent Variable:auc0toinf

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 30.609 | 1 | 30.609 | 1.866E3 | .000 | .998 | 1865.506 | 1.000 |
| | Error | .073 | 4.433 | .016[b] | | | | | |
| Period | Hypothesis | 2.576 | 1 | 2.576 | 156.763 | .000 | .975 | 156.763 | 1.000 |
| | Error | .066 | 4 | .016[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .080 | 5 | .016 | .971 | .526 | .548 | 4.854 | .141 |
| | Error | .066 | 4 | .016[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 33 Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0toinfslow - auc0toinfref | .92667 | .18608 | .07597 | .73139 | 1.12195 | 12.198 | 5 | .000 |

## Table 34
## Statistical data for Ondansetron hydrochloride fast versus reference release formulations

Dependent Variable: $C_{max}$

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 9.496 | 1 | 9.496 | 1.611E3 | .000 | .997 | 1610.678 | 1.000 |
| | Error | .024 | 4.073 | .006[b] | | | | | |
| Period | Hypothesis | .033 | 1 | .033 | 5.364 | .082 | .573 | 5.364 | .424 |
| | Error | .025 | 4 | .006[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .005 | 5 | .001 | .165 | .963 | .171 | .823 | .064 |
| | Error | .025 | 4 | .006[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 35 Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 95% Confidence Interval of the Difference | | | | |
| | | Mean | Std. Deviation | Std. Error Mean | Lower | Upper | | | |
| Pair 1 | $C_{max}$ fast - $C_{max}$ ref | .10500 | .10213 | .04169 | -.00218 | .21218 | 2.518 | 5 | .053 |

## Table 36
## Tests of Between-Subjects Effects

Dependent Variable:auc0tot

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 30.434 | 1 | 30.434 | 4.130E3 | .000 | .998 | 4129.898 | 1.000 |
| | Error | .047 | 6.406 | .007[b] | | | | | |
| Period | Hypothesis | 2.376 | 1 | 2.376 | 401.628 | .000 | .990 | 401.628 | 1.000 |
| | Error | .024 | 4 | .006[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .168 | 5 | .034 | 5.664 | .059 | .876 | 28.321 | .598 |
| | Error | .024 | 4 | .006[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 37

## Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0totfast - auc0totref | .89000 | .10159 | .04147 | .78339 | .99661 | 21.460 | 5 | .000 |

## Table 38
## Tests of Between-Subjects Effects

Dependent Variable:auc0toinf

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 30.815 | 1 | 30.815 | 4.873E3 | .000 | .999 | 4873.337 | 1.000 |
| | Error | .042 | 6.658 | .006[b] | | | | | |
| Period | Hypothesis | 2.394 | 1 | 2.394 | 485.297 | .000 | .992 | 485.297 | 1.000 |
| | Error | .020 | 4 | .005[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .157 | 5 | .031 | 6.353 | .049 | .888 | 31.764 | .648 |
| | Error | .020 | 4 | .005[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 39
## Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0toinffast - auc0toinfref | .89333 | .09245 | .03774 | .79631 | .99035 | 23.670 | 5 | .000 |

**Table 40**
**Statistical data for Dextromethorphan Hydrobromide slow versus reference release formulations**

Dependent Variable:$C_{max}$

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 41.574 | 1 | 41.574 | 2.523E4 | .000 | 1.000 | 25226.127 | 1.000 |
| | Error | .010 | 6.007 | .002[b] | | | | | |
| Period | Hypothesis | .832 | 1 | .832 | 601.542 | .000 | .993 | 601.542 | 1.000 |
| | Error | .006 | 4 | .001[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .032 | 5 | .006 | 4.636 | .081 | .853 | 23.181 | .513 |
| | Error | .006 | 4 | .001[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

**Table 41**

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | $C_{max}$ slow - $C_{max}$ ref | .52667 | .06186 | .02525 | .46175 | .59158 | 20.855 | 5 | .000 |

## Table 42

## Tests of Between-Subjects Effects

Dependent Variable:auc0tot

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 87.037 | 1 | 87.037 | 1.808E4 | .000 | 1.000 | 18083.396 | 1.000 |
| | Error | .022 | 4.619 | .005[b] | | | | | |
| Period | Hypothesis | 7.348 | 1 | 7.348 | 1.558E3 | .000 | .997 | 1557.811 | 1.000 |
| | Error | .019 | 4 | .005[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .033 | 5 | .007 | 1.388 | .386 | .634 | 6.942 | .185 |
| | Error | .019 | 4 | .005[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 43

## Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0totslow - auc0totref | 1.56500 | .09225 | .03766 | 1.46819 | 1.66181 | 41.555 | 5 | .000 |

## Table 44

## Tests of Between-Subjects Effects

Dependent Variable:auc0toinf

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 98.579 | 1 | 98.579 | 1.220E4 | .000 | 1.000 | 12200.898 | 1.000 |
| | Error | .034 | 4.204 | .008[b] | | | | | |
| Period | Hypothesis | 10.416 | 1 | 10.416 | 1.252E3 | .000 | .997 | 1252.429 | 1.000 |
| | Error | .033 | 4 | .008[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .019 | 5 | .004 | .459 | .793 | .364 | 2.293 | .091 |
| | Error | .033 | 4 | .008[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 45

## Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0toinfslow - auc0toinfref | 1.86333 | .11742 | .04794 | 1.74011 | 1.98655 | 38.872 | 5 | .000 |

## Table 46
## Statistical data for Dextromethorphan Hydrobromide fast versus reference release formulations

Dependent Variable: $C_{max}$

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 42.597 | 1 | 42.597 | 3.932E4 | .000 | 1.000 | 39317.069 | 1.000 |
| | Error | .006 | 5.375 | .001[b] | | | | | |
| Period | Hypothesis | .992 | 1 | .992 | 1.017E3 | .000 | .996 | 1017.308 | 1.000 |
| | Error | .004 | 4 | .001[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .015 | 5 | .003 | 3.113 | .147 | .796 | 15.564 | .367 |
| | Error | .004 | 4 | .001[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 47
## Paired Samples Test

| | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | Lower | Upper | | | |
| Pair 1 $C_{max}$fast - $C_{max}$ref | .57500 | .05505 | .02247 | .51723 | .63277 | 25.587 | 5 | .000 |

## Table 48
## Tests of Between-Subjects Effects

Dependent Variable:auc0tot

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 90.935 | 1 | 90.935 | 6.422E3 | .000 | .999 | 6422.223 | 1.000 |
| | Error | .061 | 4.323 | .014[b] | | | | | |
| Period | Hypothesis | 8.824 | 1 | 8.824 | 614.177 | .000 | .994 | 614.177 | 1.000 |
| | Error | .057 | 4 | .014[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .052 | 5 | .010 | .726 | .639 | .476 | 3.629 | .117 |
| | Error | .057 | 4 | .014[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 49
## Paired Samples Test

| | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | Lower | Upper | | | |
| Pair 1 auc0totfast - auc0totref | 1.71500 | .15162 | .06190 | 1.55588 | 1.87412 | 27.706 | 5 | .000 |

## Table 50
## Tests of Between-Subjects Effects

Dependent Variable:auc0toinf

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 101.173 | 1 | 101.173 | 9.956E3 | .000 | 1.000 | 9956.292 | 1.000 |
| | Error | .043 | 4.238 | .010[b] | | | | | |
| Period | Hypothesis | 11.643 | 1 | 11.643 | 1.118E3 | .000 | .996 | 1117.699 | 1.000 |
| | Error | .042 | 4 | .010[c] | | | | | |
| Sequence | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Treatment | Hypothesis | .000 | 0 | . | . | . | . | . | . |
| | Error | . | . | .[d] | | | | | |
| Subject | Hypothesis | .028 | 5 | .006 | .535 | .746 | .401 | 2.675 | .098 |
| | Error | .042 | 4 | .010[c] | | | | | |

[a]Computed using alpha = .05

[b].053 MS(Subject) + MS(Error)

[c]MS(Error)

[d]Cannot compute the appropriate error term using Satterthwaite's method.

## Table 51
## Paired Samples Test

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | auc0toinffast - auc0toinfref | 1.97000 | .12992 | .05304 | 1.83365 | 2.10635 | 37.141 | 5 | .000 |

**Table 52 Cumulative percentage dissolved at 50 rpm for the Ondansetron hydrochloride test formulations**

| Time (h) | Square root of time(h) | pH 1.2 buffer Formulation | | pH 4.5 buffer Formulation | | pH 6.8 buffer Formulation | | pH 5.5 buffer Formulation | | pH 7.4 buffer Formulation | | Water Formulation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **0.50** | 0.71 | 30.70 | 36.59 | 9.23 | 10.26 | 7.29 | 6.95 | 13.13 | 23.54 | 8.65 | 13.72 | 15.64 | 27.68 |
| **1.00** | 1.00 | 50.84 | 68.04 | 15.88 | 27.99 | 16.78 | 17.67 | 17.86 | 29.08 | 18.97 | 23.54 | 24.58 | 34.68 |
| **1.50** | 1.22 | 53.51 | 80.05 | 19.78 | 36.35 | 15.20 | 21.64 | 23.08 | 39.83 | 27.65 | 32.78 | 32.83 | 39.35 |
| **2.00** | 1.41 | 62.27 | 85.67 | 25.68 | 48.77 | 23.58 | 34.75 | 26.58 | 48.44 | 37.03 | 42.11 | 38.93 | 47.77 |
| **2.50** | 1.58 | 63.16 | 93.52 | 28.62 | 53.28 | 36.55 | 48.17 | 31.62 | 52.20 | 43.25 | 53.38 | 40.87 | 54.24 |
| **3.00** | 1.73 | 71.59 | 99.63 | 32.48 | 64.69 | 42.50 | 53.33 | 37.62 | 66.15 | 49.97 | 62.67 | 44.86 | 65.56 |
| **4.00** | 2.00 | 90.95 | 102.90 | 43.40 | 73.52 | 46.80 | 66.14 | 53.49 | 72.80 | 52.14 | 72.89 | 51.51 | 79.29 |
| **6.00** | 2.45 | 106.44 | 103.12 | 55.12 | 80.64 | 66.93 | 73.00 | 59.00 | 80.67 | 58.56 | 79.62 | 61.48 | 85.79 |
| **8.00** | 2.83 | 108.22 | 104.26 | 60.85 | 84.77 | 71.06 | 79.72 | 66.48 | 88.09 | 64.91 | 81.79 | 70.74 | 94.14 |
| **12.00** | 3.46 | 109.92 | 104.73 | 78.99 | 95.63 | 78.33 | 84.05 | 74.83 | 93.73 | 72.69 | 95.09 | 78.01 | 102.82 |
| **18.00** | 4.24 | 109.15 | 104.80 | 89.79 | 98.44 | 86.05 | 95.88 | 87.76 | 100.42 | 80.10 | 97.76 | 94.15 | 103.31 |
| **24.00** | 4.90 | 110.17 | 104.45 | 94.28 | 99.86 | 93.91 | 99.32 | 93.11 | 101.41 | 85.87 | 99.14 | 100.15 | 103.09 |

**Table 53 Cumulative percentage dissolved at 75 rpm for the Ondansetron hydrochloride test formulations**

| Time (h) | Square root of time(h) | pH 1.2 buffer | | pH 4.5 buffer | | pH 6.8 buffer | | pH 5.5 buffer | | pH 7.4 buffer | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Formulation | | Formulation | | Formulation | | Formulation | | Formulation | | Formulation | |
| | | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **0.50** | 0.71 | 35.22 | 43.71 | 13.67 | 16.88 | 11.48 | 9.64 | 17.26 | 19.64 | 10.56 | 18.32 | 20.56 | 37.62 |
| **1.00** | 1.00 | 60.69 | 73.78 | 19.00 | 29.82 | 22.04 | 22.40 | 26.25 | 33.92 | 24.67 | 27.94 | 27.04 | 44.16 |
| **1.50** | 1.22 | 68.48 | 88.33 | 23.27 | 46.71 | 34.74 | 30.39 | 35.01 | 48.14 | 32.92 | 44.08 | 35.76 | 56.86 |
| **2.00** | 1.41 | 78.83 | 98.54 | 29.96 | 53.38 | 48.87 | 39.80 | 42.16 | 56.56 | 44.09 | 51.01 | 44.18 | 64.37 |
| **2.50** | 1.58 | 94.08 | 99.87 | 34.82 | 59.97 | 52.33 | 54.44 | 52.59 | 67.62 | 51.86 | 58.42 | 46.56 | 78.34 |
| **3.00** | 1.73 | 97.56 | 100.52 | 41.49 | 67.15 | 69.54 | 72.79 | 59.92 | 70.61 | 58.16 | 67.24 | 50.43 | 83.69 |
| **4.00** | 2.00 | 100.08 | 101.81 | 54.01 | 75.70 | 73.67 | 80.72 | 63.45 | 79.04 | 63.57 | 73.82 | 53.91 | 89.72 |
| **6.00** | 2.45 | 101.68 | 102.40 | 63.29 | 88.01 | 75.36 | 86.89 | 69.30 | 84.23 | 72.93 | 83.95 | 70.10 | 93.44 |
| **8.00** | 2.83 | 102.82 | 103.34 | 71.89 | 94.13 | 86.12 | 92.84 | 74.44 | 92.34 | 79.77 | 89.48 | 79.10 | 99.86 |
| **12.00** | 3.46 | 103.12 | 103.52 | 90.13 | 100.75 | 91.76 | 99.15 | 96.28 | 102.86 | 86.13 | 97.16 | 96.66 | 103.55 |
| **18.00** | 4.24 | 103.24 | 101.42 | 94.02 | 101.18 | 97.33 | 100.51 | 97.58 | 103.00 | 89.70 | 99.09 | 99.32 | 102.30 |
| **24.00** | 4.90 | 104.49 | 103.55 | 98.82 | 102.13 | 99.88 | 103.42 | 99.08 | 103.11 | 94.32 | 100.47 | 100.17 | 103.87 |

**Table 54 Similarity factors for Ondansetron hydrochloride modified release dosage forms in various dissolution conditions**

| S.No | pH | Conditions | Formulation | Similarity factor ($f_2$) |
|---|---|---|---|---|
| 1 | pH 1.2 buffer | 50 rpm | Fast versus slow | 38.22 |
| 2 | pH 1.2 buffer | 75 rpm | Fast versus slow | 51.06 |
| 3 | pH 4.5 buffer | 50 rpm | Fast versus slow | 34.23 |
| 4 | pH 4.5 buffer | 75 rpm | Fast versus slow | 36.19 |
| 5 | pH 6.8 buffer | 50 rpm | Fast versus slow | 51.18 |
| 6 | pH 6.8 buffer | 75 rpm | Fast versus slow | 60.97 |
| 7 | pH 5.5 buffer | 50 rpm | Fast versus slow | 36.56 |
| 8 | pH 5.5 buffer | 75 rpm | Fast versus slow | 46.40 |
| 9 | Distilled Water | 50 rpm | Fast versus slow | 52.23 |
| 10 | Distilled Water | 75 rpm | Fast versus slow | 70.52 |
| 11 | pH 7.4 buffer | 50 rpm | Fast versus slow | 38.93 |
| 12 | pH 7.4 buffer | 75 rpm | Fast versus slow | 42.54 |

**Figure 28**

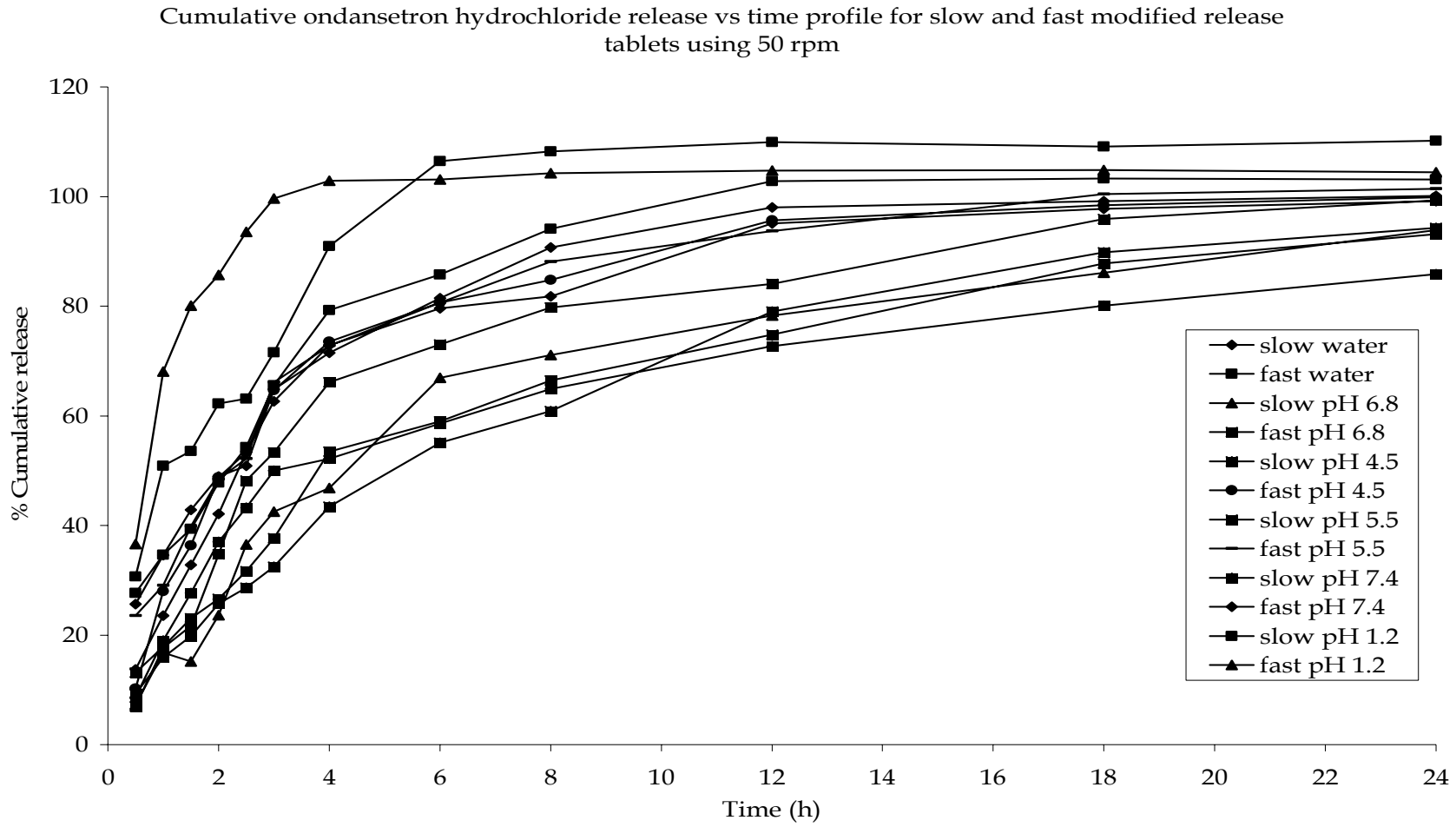

Cumulative ondansetron hydrochloride release vs time profile for slow and fast modified release tablets using 50 rpm
% Cumulative release
Time (h)
0
20
40
60
80
100
120
0
2
4
6
8
10
12
14
16
18
20
22
24
slow water
fast water
slow pH 6.8
fast pH 6.8
slow pH 4.5
fast pH 4.5
slow pH 5.5
fast pH 5.5
slow pH 7.4
fast pH 7.4
slow pH 1.2
fast pH 1.2

**Figure 29**

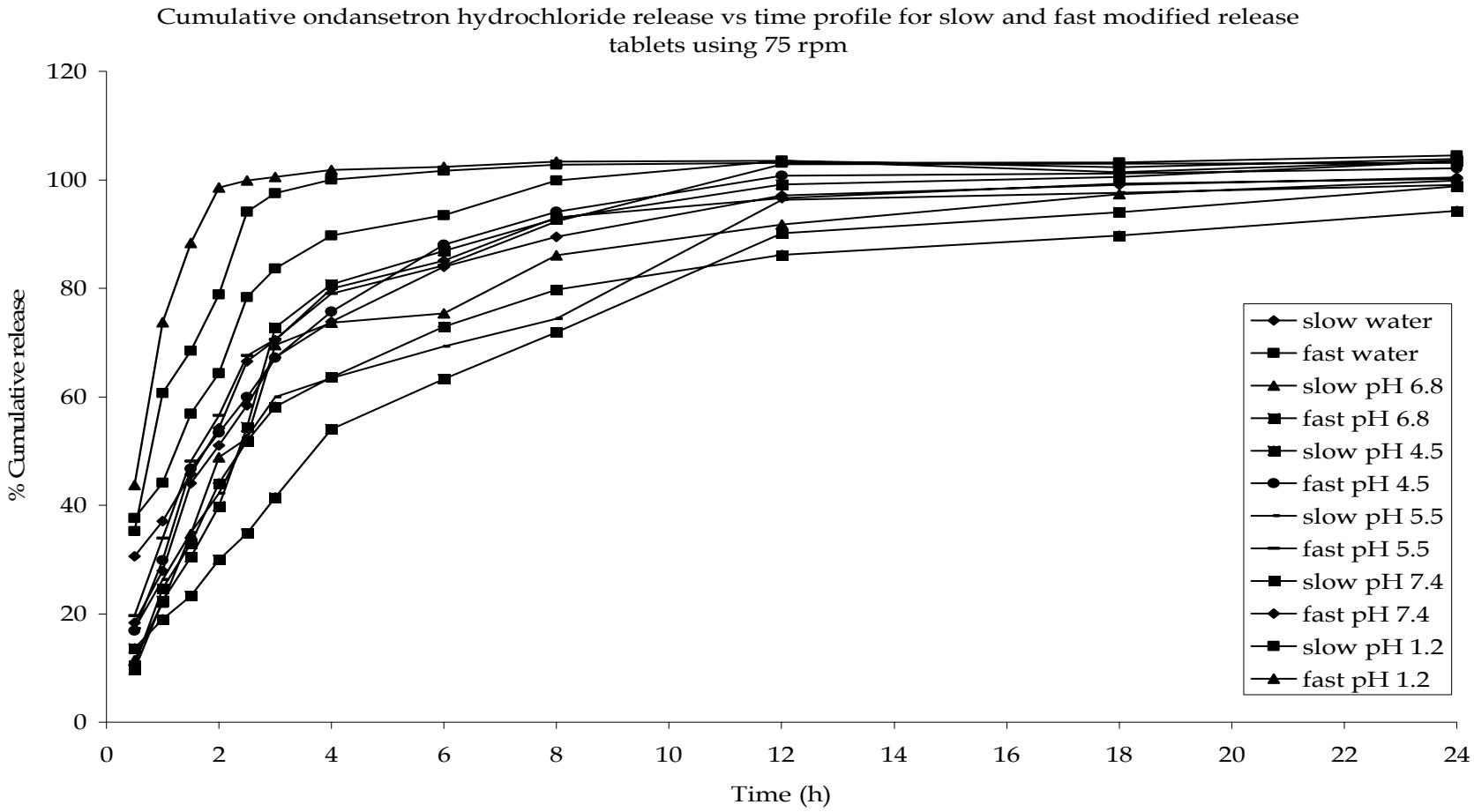

Cumulative ondansetron hydrochloride release vs time profile for slow and fast modified release tablets using 75 rpm
% Cumulative release
Time (h)
0
20
40
60
80
100
120
0
2
4
6
8
10
12
14
16
18
20
22
24
slow water
fast water
slow pH 6.8
fast pH 6.8
slow pH 4.5
fast pH 4.5
slow pH 5.5
fast pH 5.5
slow pH 7.4
fast pH 7.4
slow pH 1.2
fast pH 1.2

**Table 55 IVIVC model linear regression of % absorbed versus % dissolved for Ondansetron hydrochloride tablets using pH 4.5 at 50 rpm**

| Time | Percentage dissolved (pH 4.5) | | Percentage absorbed | |
|---|---|---|---|---|
| | slow | fast | slow | fast |
| **0.0** | **0.0** | **0.0** | **0.00** | **0.00** |
| 0.5 | 9.23 | 10.26 | 8.59 | 8.56 |
| 1 | 15.88 | 27.99 | 17.96 | 26.83 |
| 1.5 | 19.78 | 36.35 | 28.47 | 40.72 |
| 2 | 25.68 | 53.77 | 40.44 | 55.85 |
| 2.5 | 46.62 | 63.28 | 54.28 | 68.89 |
| 3 | 62.48 | 74.69 | 72.72 | 82.98 |
| 4 | 73.4 | 83.52 | 95.30 | 103.11 |
| 6 | 85.12 | 92.64 | 111.51 | 110.44 |
| 8 | 90.85 | 98.77 | 103.37 | 103.74 |
| 12 | 98.99 | 100.63 | 92.64 | 85.04 |
| 18 | 99.79 | 100.44 | 88.40 | 91.43 |
| 24 | 100.28 | 102.86 | 81.64 | 85.18 |

**Table 56 IVIVC model linear regression of % absorbed versus % dissolved for Ondansetron hydrochloride tablets using pH 4.5 at 75 rpm**

| Time | Percentage dissolved (pH 4.5) | | Percentage absorbed | |
|---|---|---|---|---|
| | slow | fast | slow | fast |
| 0.0 | 0.0 | 0.0 | 0.00 | 0.00 |
| 0.5 | 13.67 | 16.88 | 8.59 | 8.56 |
| 1 | 18.92 | 29.73 | 17.96 | 26.83 |
| 1.5 | 23.16 | 46.54 | 28.47 | 40.72 |
| 2 | 29.83 | 53.12 | 40.44 | 55.85 |
| 2.5 | 34.65 | 59.67 | 54.28 | 68.89 |
| 3 | 41.30 | 66.82 | 72.72 | 82.98 |
| 4 | 53.78 | 75.33 | 95.30 | 103.11 |
| 6 | 62.99 | 87.59 | 111.51 | 110.44 |
| 8 | 71.54 | 93.64 | 103.37 | 103.74 |
| 12 | 89.73 | 100.23 | 92.64 | 85.04 |
| 18 | 93.52 | 100.62 | 88.40 | 91.43 |
| 24 | 98.30 | 101.57 | 81.64 | 85.18 |

**Figure 30**

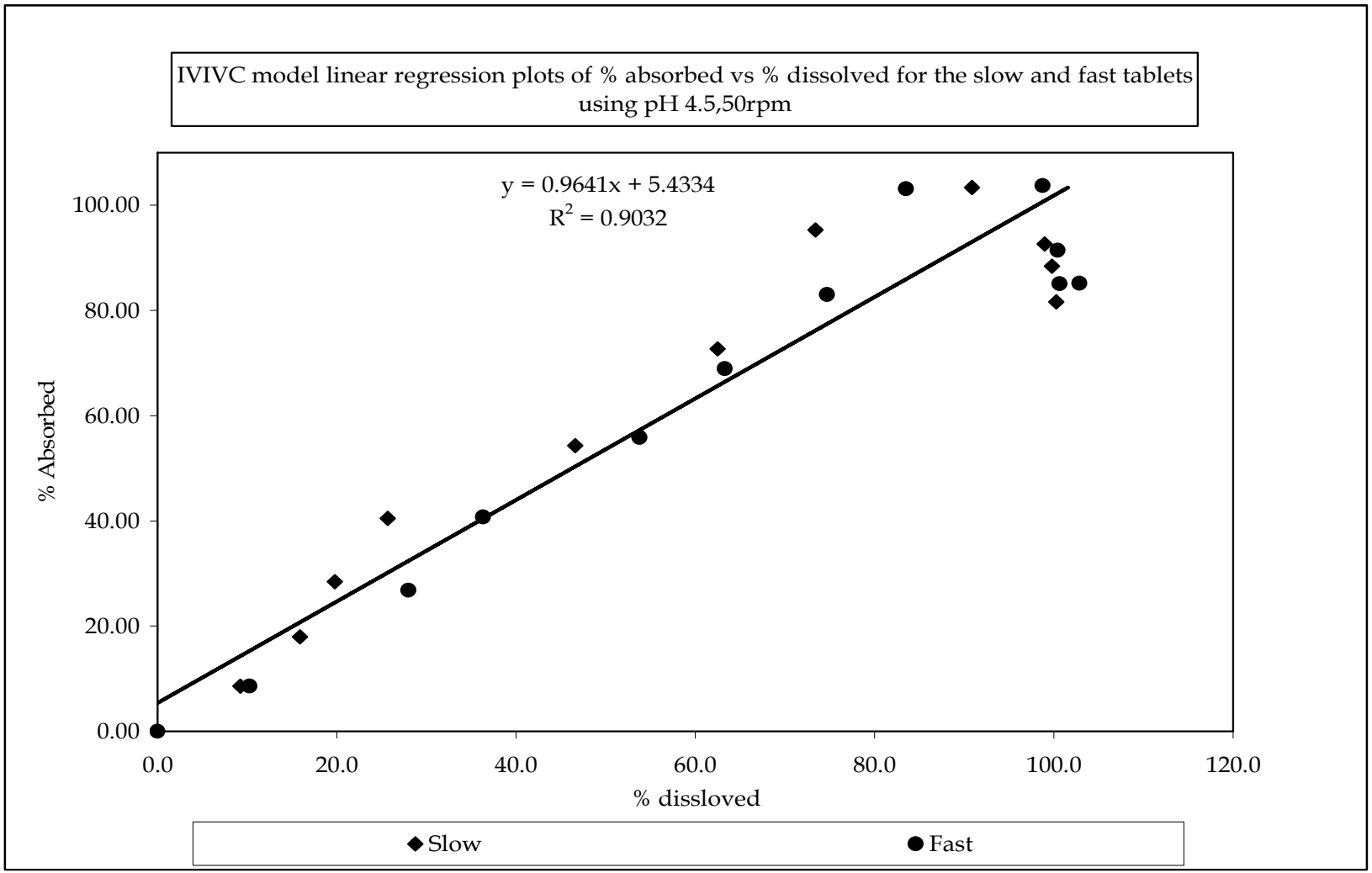

IVIVC model linear regression plots of % absorbed vs % dissolved for the slow and fast tablets using pH 4.5,50rpm
y = 0.9641x + 5.4334
R2 = 0.9032
% Absorbed
% dissloved
0.00
20.00
40.00
60.00
80.00
100.00
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Slow
Fast

**Figure 31**

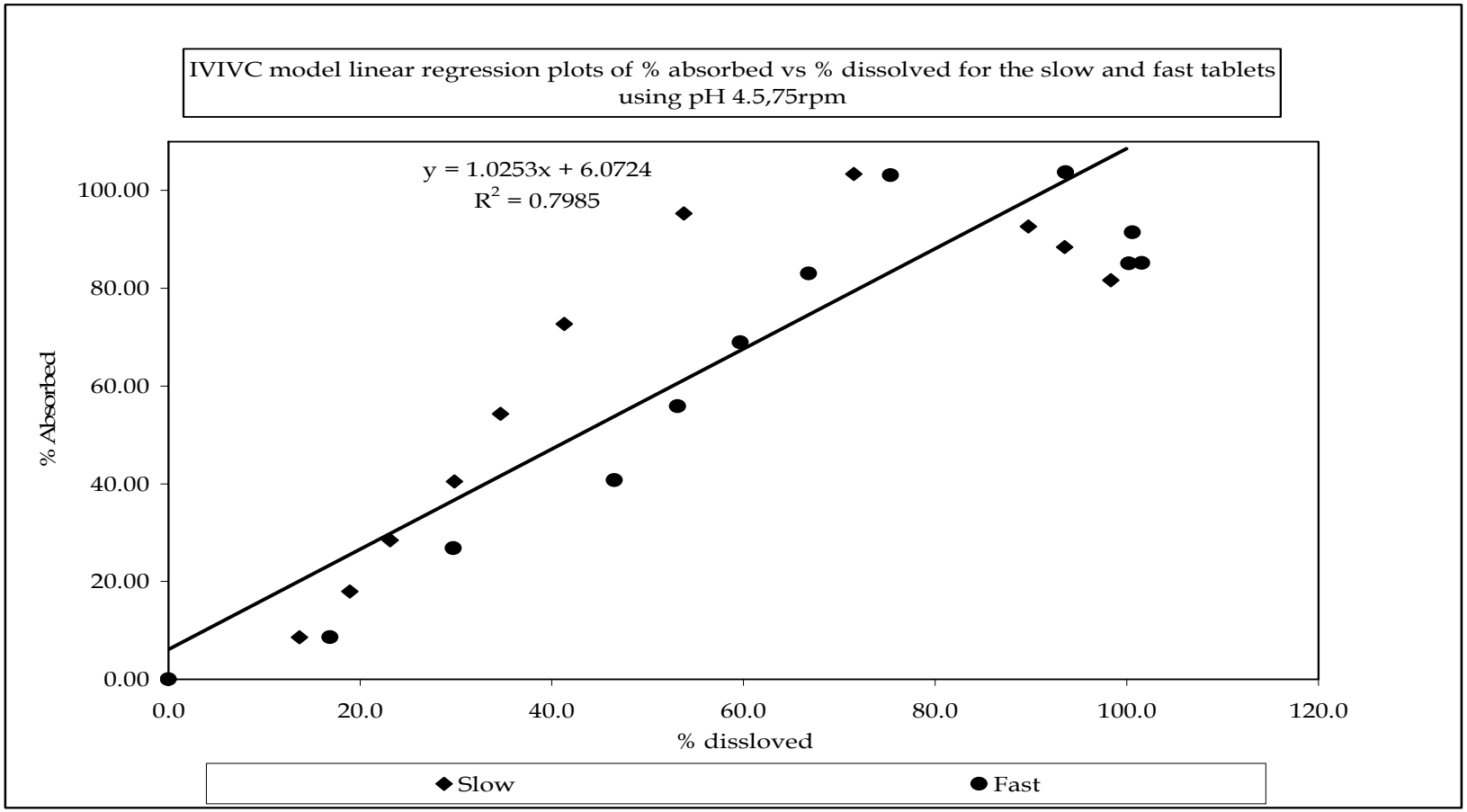

IVIVC model linear regression plots of % absorbed vs % dissolved for the slow and fast tablets using pH 4.5,75rpm
y = 1.0253x + 6.0724
R2 = 0.7985
% Absorbed
100.00
80.00
60.00
40.00
20.00
0.00
0.0
20.0
40.0
60.0
80.0
100.0
120.0
% dissloved
Slow
Fast

**Figure 32**

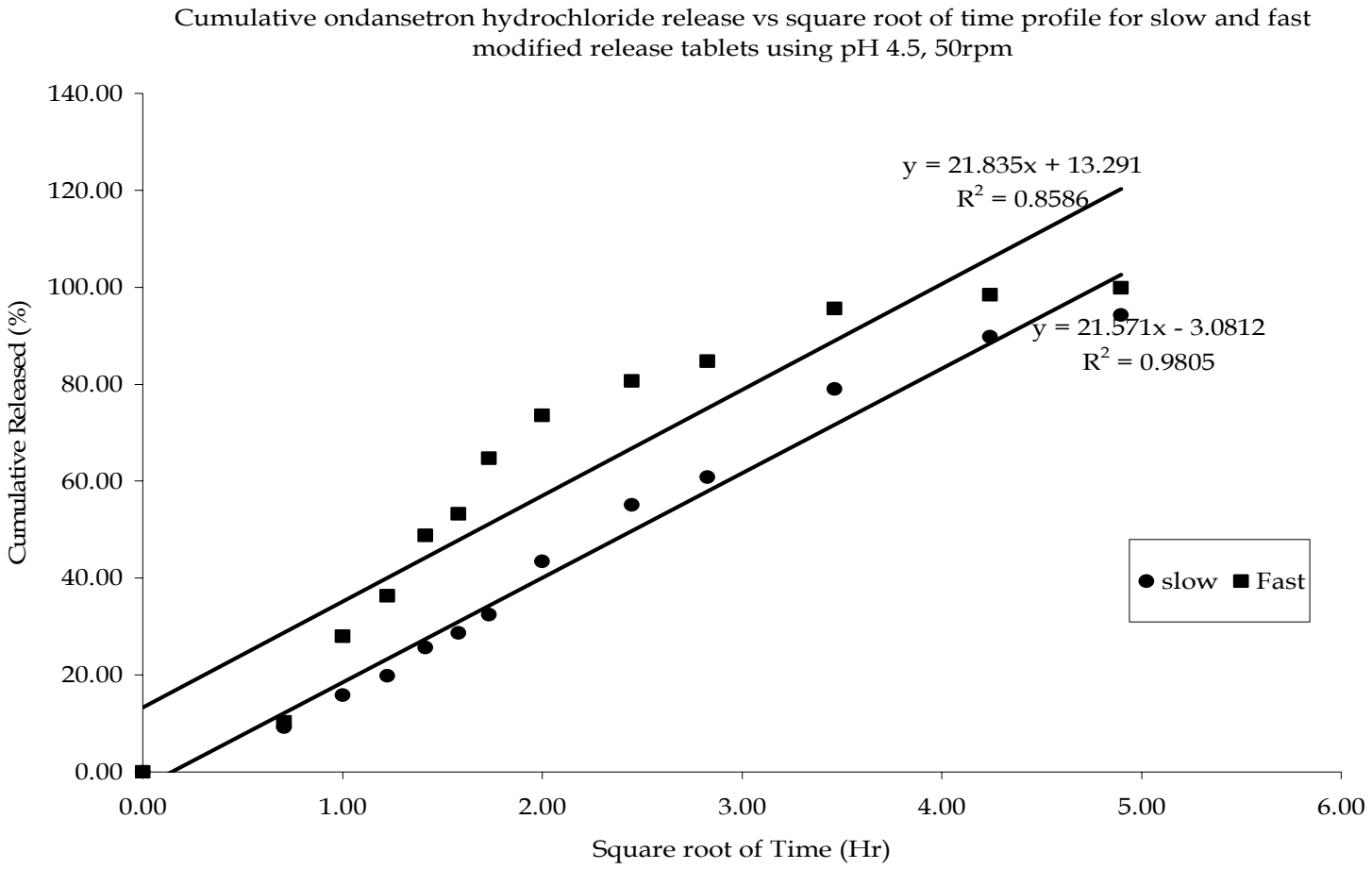

Cumulative ondansetron hydrochloride release vs square root of time profile for slow and fast modified release tablets using pH 4.5, 50rpm
y = 21.835x + 13.291
R2 = 0.8586
y = 21.571x - 3.0812
R2 = 0.9805
slow
Fast
Cumulative Released (%)
Square root of Time (Hr)
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00

**Figure 33**

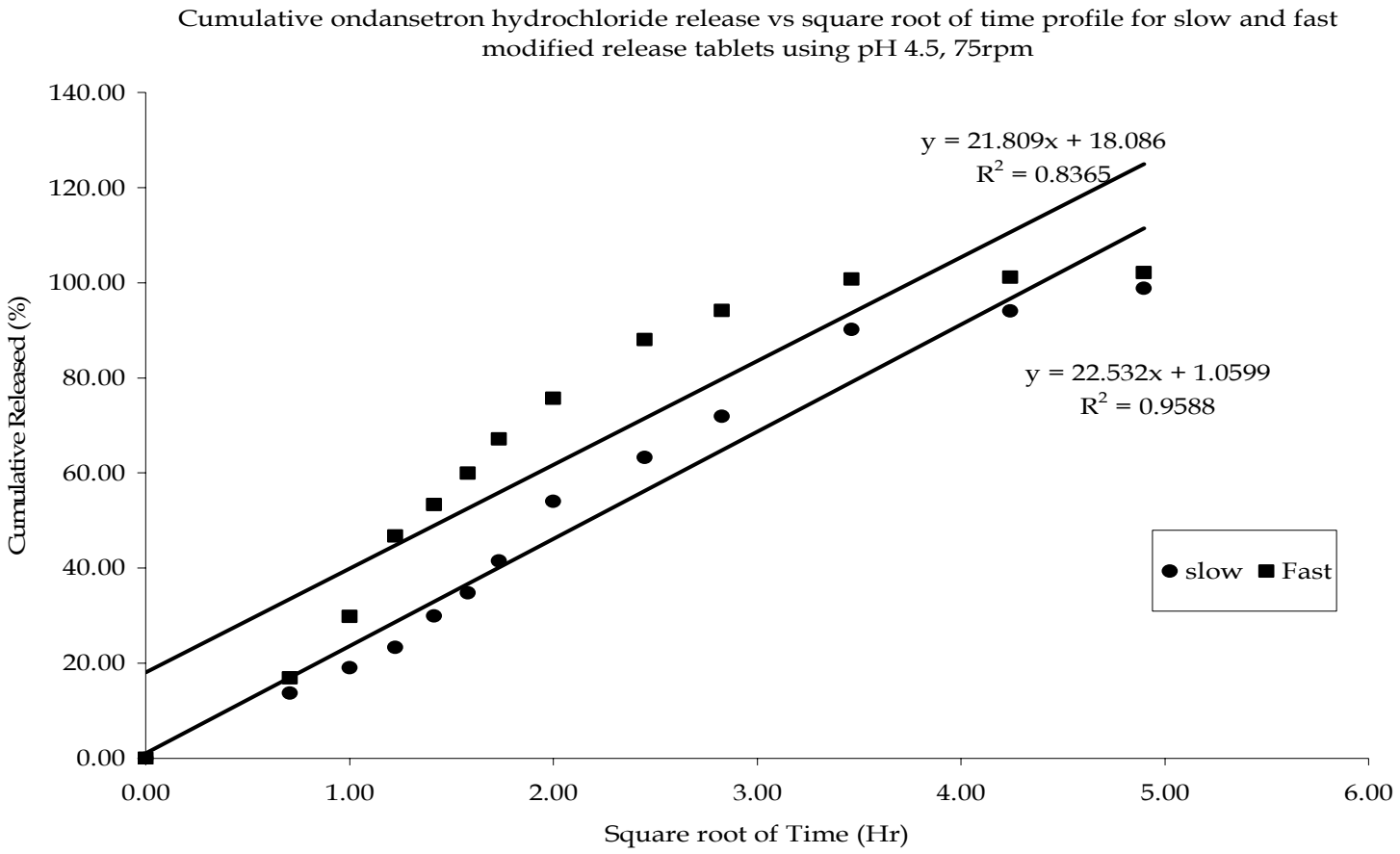

Cumulative ondansetron hydrochloride release vs square root of time profile for slow and fast modified release tablets using pH 4.5, 75rpm
y = 21.809x + 18.086
R2 = 0.8365
y = 22.532x + 1.0599
R2 = 0.9588
slow
Fast
Cumulative Released (%)
Square root of Time (Hr)
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00

**Figure 34**

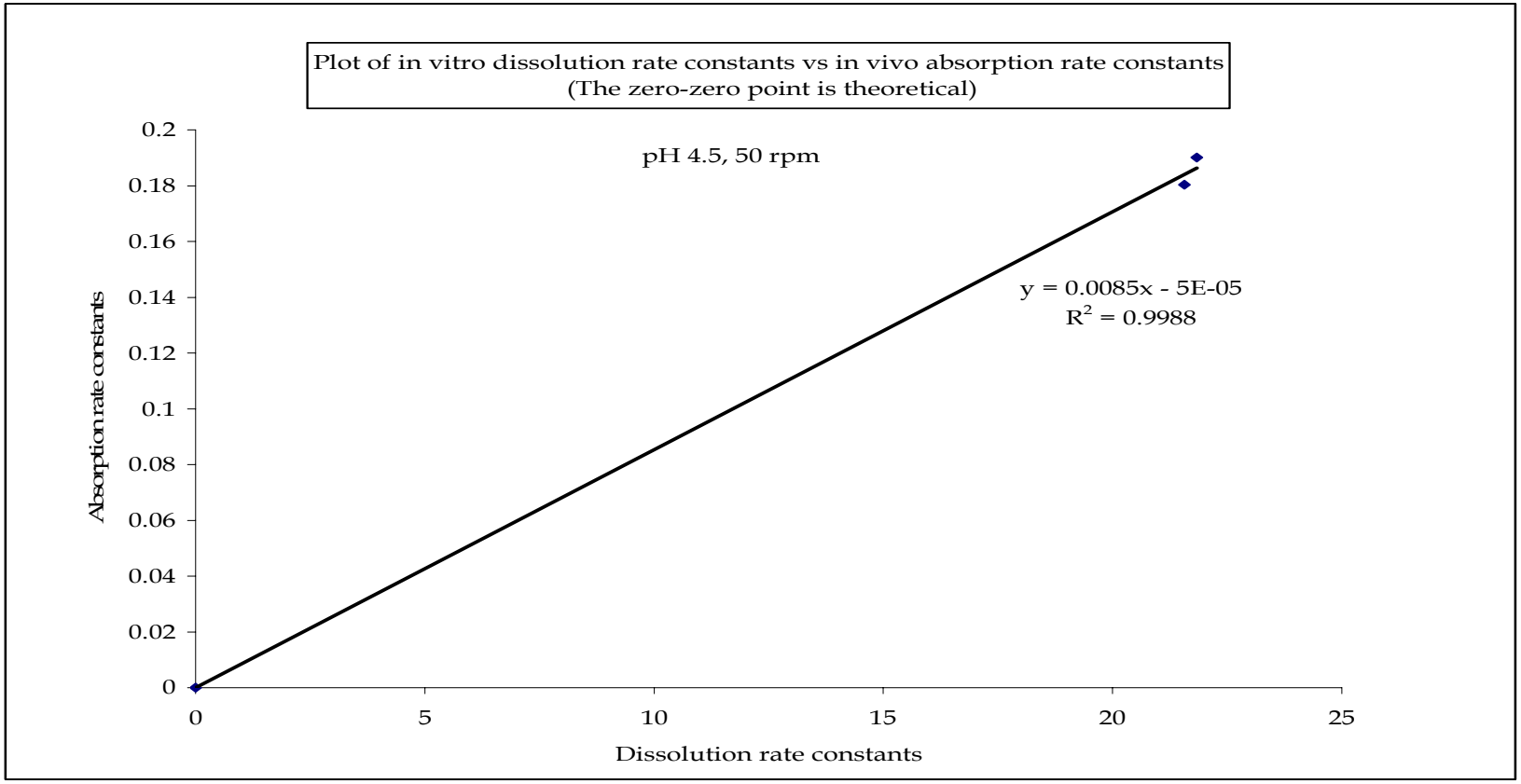

Plot of in vitro dissolution rate constants vs in vivo absorption rate constants
(The zero-zero point is theoretical)
pH 4.5, 50 rpm
y = 0.0085x - 5E-05
R2 = 0.9988
Absorption rate constants
Dissolution rate constants
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
0
5
10
15
20
25

**Table 57 Observed and IVIVC model predicted $C_{max}$ and AUC values for Ondansetron hydrochloride**

| Time (Hours) | Slow formulation | | Fast formulation | |
|---|---|---|---|---|
| | Fraction observed | Fraction predicted | Fraction observed | Fraction observed |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.50 | 4.96 | 1.35 | 5.09 | 1.45 |
| 1.00 | 10.13 | 5.01 | 15.69 | 8.07 |
| 1.50 | 15.71 | 10.56 | 23.16 | 16.09 |
| 2.00 | 21.87 | 17.78 | 30.99 | 25.86 |
| 2.50 | 27.66 | 27.74 | 35.51 | 36.07 |
| 3.00 | 35.50 | 39.04 | 40.19 | 44.19 |
| 4.00 | 41.40 | 46.22 | 42.43 | 46.12 |
| 6.00 | 42.83 | 43.16 | 38.64 | 36.93 |
| 8.00 | 30.90 | 26.35 | 28.51 | 22.58 |
| 12.00 | 19.67 | 13.83 | 12.84 | 9.81 |
| 18.00 | 8.88 | 6.10 | 9.74 | 7.63 |
| 24.00 | 1.79 | 1.03 | 2.22 | 0.25 |
| **AUC** | **464.20** | **404.04** | **441.13** | **383.23** |
| **$C_{max}$** | **42.83** | **46.22** | **42.43** | **46.12** |

**Table 58 Prediction errors (%) associated with $C_{max}$ and AUC for Ondansetron hydrochloride**

| Formulation | $C_{max}$ | AUC |
|---|---|---|
| Slow | -7.91 | 8.44 |
| Fast | -8.70 | 9.27 |
| Average | -8.31 | 8.86 |

**Figure 35**

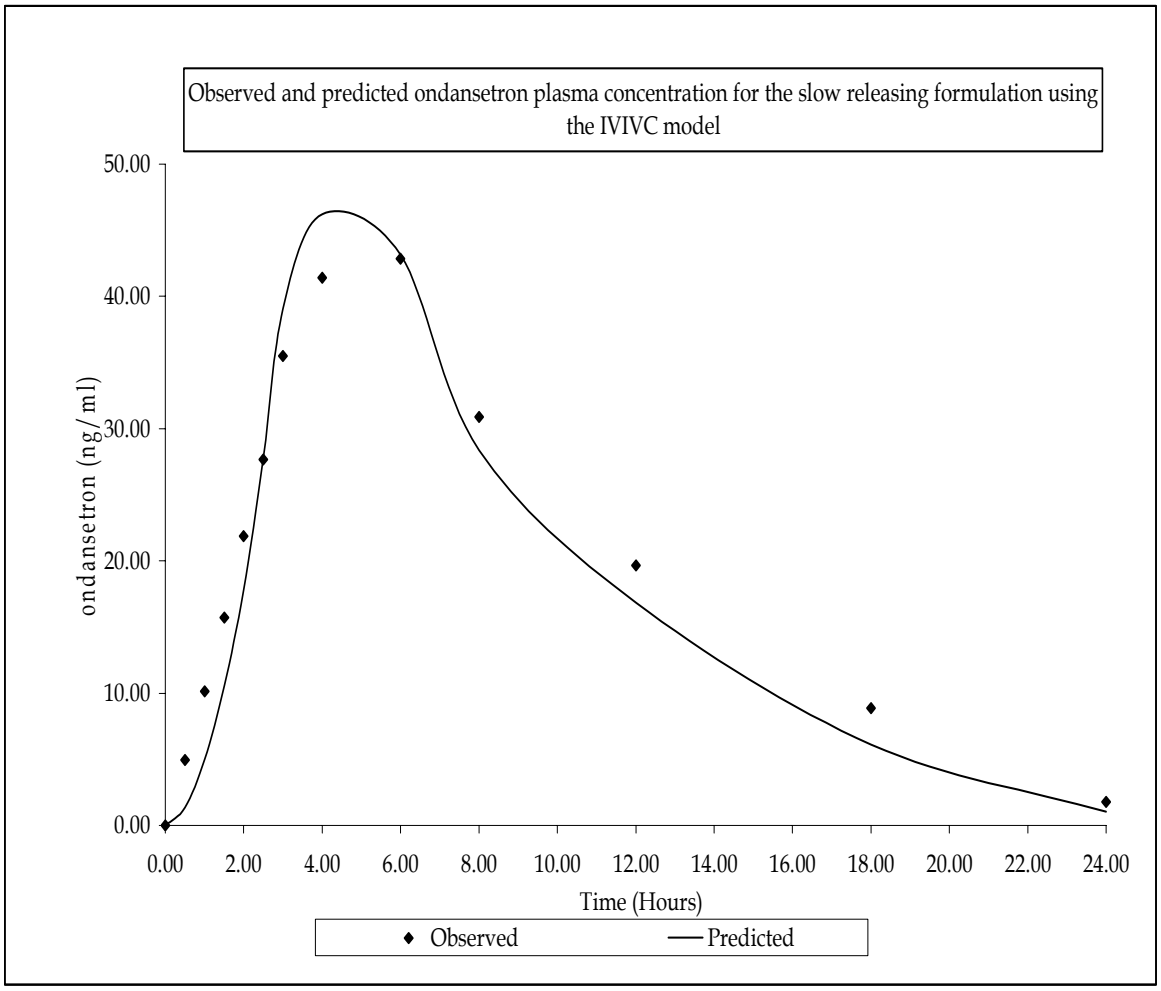

Observed and predicted ondansetron plasma concentration for the slow releasing formulation using the IVIVC model
ondansetron (ng/ml)
50.00
40.00
30.00
20.00
10.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Time (Hours)
Observed
Predicted

**Figure 36**

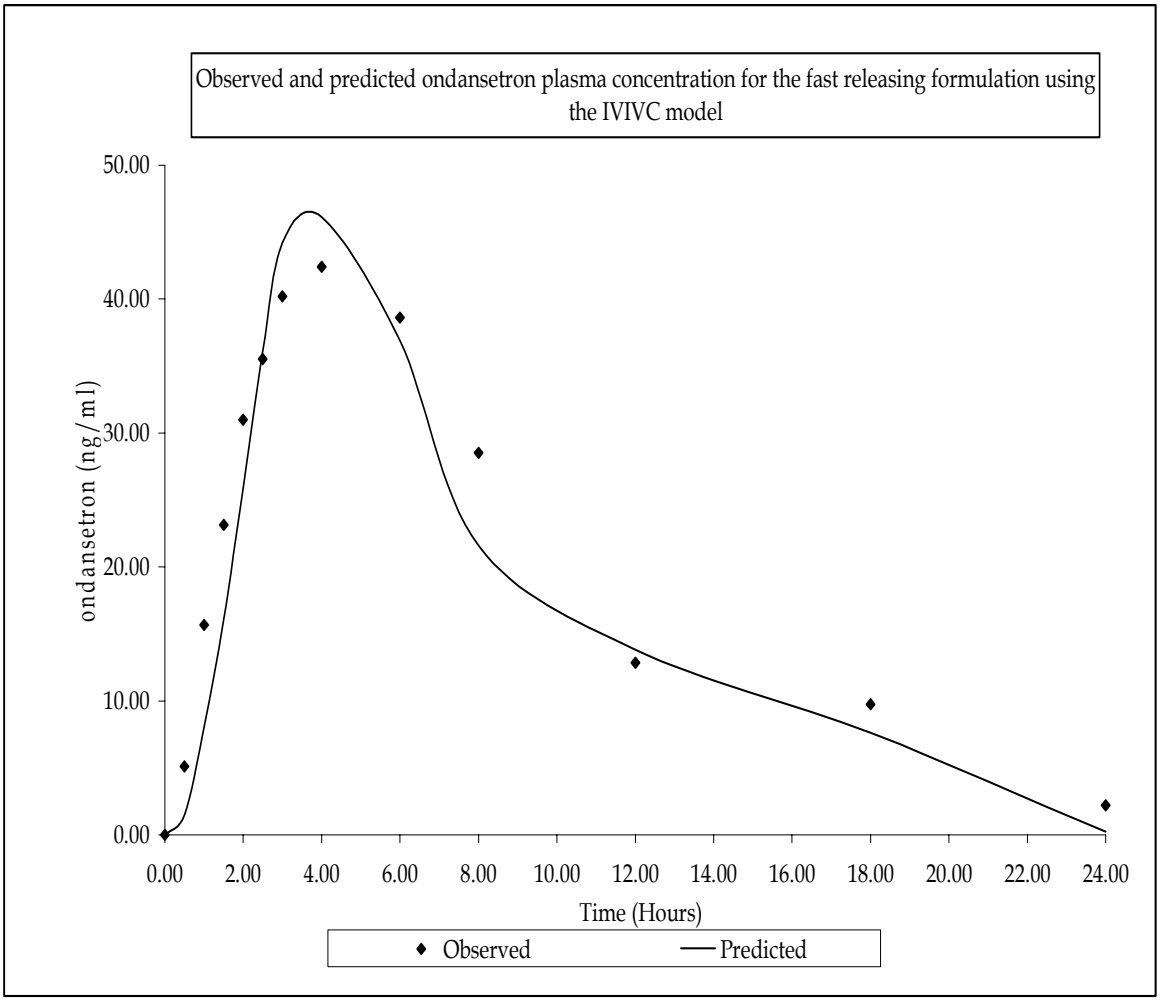

Observed and predicted ondansetron plasma concentration for the fast releasing formulation using the IVIVC model
ondansetron (ng/ml)
50.00
40.00
30.00
20.00
10.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Time (Hours)
Observed
Predicted

**Table 59 Cumulative percentage dissolved at 50 rpm for the Dextromethorphan hydrobromide test formulations**

| Time (h) | Square root of time(h) | pH 1.2 buffer | | pH 4.5 buffer | | pH 6.8 buffer | | pH 5.5 buffer | | pH 7.4 buffer | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Formulation | | Formulation | | Formulation | | Formulation | | Formulation | |
| | | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **0.50** | 0.71 | 13.93 | 15.01 | 10.58 | 9.31 | 9.84 | 17.63 | 9.69 | 9.54 | 9.31 | 13.98 |
| **1.00** | 1.00 | 18.29 | 23.73 | 15.26 | 33.28 | 15.96 | 31.10 | 17.24 | 22.37 | 15.26 | 33.28 |
| **1.50** | 1.22 | 21.82 | 32.01 | 21.44 | 33.28 | 21.49 | 30.21 | 23.66 | 28.54 | 21.44 | 45.88 |
| **2.00** | 1.41 | 26.11 | 33.10 | 27.32 | 45.88 | 32.39 | 38.81 | 28.16 | 33.41 | 27.32 | 53.67 |
| **2.50** | 1.58 | 28.93 | 38.19 | 38.93 | 53.67 | 45.67 | 54.25 | 35.58 | 40.55 | 38.93 | 56.51 |
| **3.00** | 1.73 | 31.69 | 40.79 | 39.42 | 56.51 | 46.09 | 55.35 | 39.15 | 50.88 | 39.42 | 58.63 |
| **4.00** | 2.00 | 36.12 | 43.66 | 44.30 | 58.63 | 58.57 | 56.31 | 55.16 | 56.86 | 44.30 | 71.27 |
| **6.00** | 2.45 | 53.85 | 58.87 | 59.89 | 71.27 | 80.55 | 61.36 | 63.57 | 63.26 | 59.89 | 87.66 |
| **8.00** | 2.83 | 72.47 | 70.95 | 62.02 | 82.48 | 81.65 | 66.12 | 74.33 | 68.16 | 68.92 | 94.07 |
| **12.00** | 3.46 | 73.81 | 72.97 | 70.34 | 87.66 | 87.53 | 78.08 | 80.10 | 70.58 | 81.85 | 99.09 |
| **18.00** | 4.24 | 77.60 | 77.94 | 79.68 | 89.36 | 93.54 | 92.57 | 82.99 | 77.13 | 88.90 | 102.95 |
| **24.00** | 4.90 | 84.56 | 86.10 | 92.10 | 95.83 | 94.85 | 102.51 | 89.15 | 83.74 | 94.78 | 103.44 |

**Table 60 Cumulative percentage dissolved at 75 rpm for the Dextromethorphan hydrobromide test formulations**

| Time (h) | Square root of time(h) | pH 1.2 buffer | | pH 4.5 buffer | | pH 6.8 buffer | | pH 5.5 buffer | | pH 7.4 buffer | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Formulation | | Formulation | | Formulation | | Formulation | | Formulation | |
| | | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast | Slow | Fast |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **0.50** | 0.71 | 21.93 | 11.79 | 9.04 | 11.04 | 16.83 | 11.89 | 10.31 | 11.88 | 16.83 | 11.89 |
| **1.00** | 1.00 | 30.57 | 20.52 | 14.94 | 16.49 | 29.06 | 19.18 | 15.71 | 16.91 | 29.06 | 19.18 |
| **1.50** | 1.22 | 34.27 | 28.25 | 23.08 | 23.89 | 38.42 | 26.98 | 18.73 | 20.64 | 38.42 | 26.98 |
| **2.00** | 1.41 | 40.29 | 34.11 | 24.47 | 29.54 | 42.72 | 31.16 | 21.60 | 24.65 | 42.72 | 31.16 |
| **2.50** | 1.58 | 43.36 | 42.77 | 29.24 | 38.10 | 57.67 | 47.85 | 31.89 | 31.19 | 57.67 | 47.85 |
| **3.00** | 1.73 | 50.34 | 46.96 | 33.46 | 45.45 | 59.83 | 49.90 | 36.84 | 44.32 | 59.83 | 49.90 |
| **4.00** | 2.00 | 55.67 | 49.35 | 52.34 | 61.65 | 62.40 | 62.54 | 47.09 | 63.56 | 62.40 | 62.54 |
| **6.00** | 2.45 | 66.89 | 61.12 | 68.64 | 68.92 | 68.34 | 75.15 | 55.06 | 68.10 | 68.34 | 75.15 |
| **8.00** | 2.83 | 80.79 | 75.47 | 87.01 | 84.23 | 71.63 | 81.61 | 63.48 | 78.58 | 71.63 | 81.61 |
| **12.00** | 3.46 | 84.56 | 85.24 | 90.66 | 92.81 | 81.27 | 87.71 | 66.91 | 94.75 | 74.74 | 84.98 |
| **18.00** | 4.24 | 87.41 | 90.92 | 94.70 | 103.02 | 86.53 | 96.98 | 77.49 | 99.26 | 86.73 | 98.28 |
| **24.00** | 4.90 | 95.87 | 95.51 | 98.05 | 105.76 | 95.31 | 105.62 | 89.03 | 101.36 | 93.02 | 101.30 |

**Table 61 Similarity factors for Dextromethorphan hydrobromide modified release dosage forms in various dissolution conditions**

| S.No | pH | Conditions | Formulation | Similarity factor ($f_2$) |
|---|---|---|---|---|
| 1 | pH 1.2 buffer | 50 rpm | Fast versus slow | 60.61 |
| 2 | pH 1.2 buffer | 75 rpm | Fast versus slow | 61.49 |
| 3 | pH 4.5 buffer | 50 rpm | Fast versus slow | 42.06 |
| 4 | pH 4.5 buffer | 75 rpm | Fast versus slow | 59.62 |
| 5 | pH 6.8 buffer | 50 rpm | Fast versus slow | 48.79 |
| 6 | pH 6.8 buffer | 75 rpm | Fast versus slow | 52.02 |
| 7 | pH 5.5 buffer | 50 rpm | Fast versus slow | 60.72 |
| 8 | pH 5.5 buffer | 75 rpm | Fast versus slow | 43.64 |
| 9 | pH 7.4 buffer | 50 rpm | Fast versus slow | 34.44 |
| 10 | pH 7.4 buffer | 75 rpm | Fast versus slow | 41.49 |

**Figure 37**

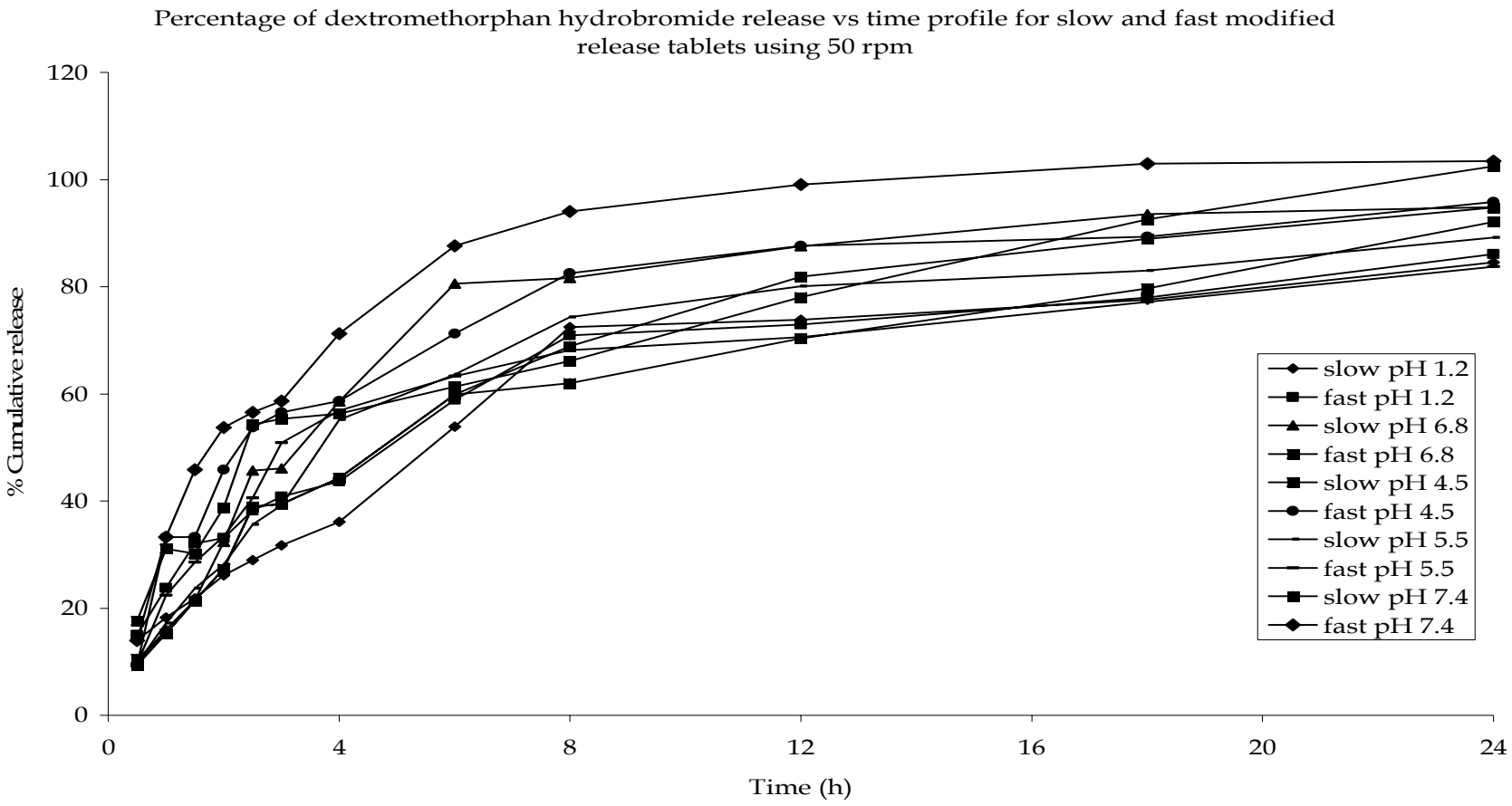

Percentage of dextromethorphan hydrobromide release vs time profile for slow and fast modified release tablets using 50 rpm
% Cumulative release
Time (h)
0
20
40
60
80
100
120
0
4
8
12
16
20
24
slow pH 1.2
fast pH 1.2
slow pH 6.8
fast pH 6.8
slow pH 4.5
fast pH 4.5
slow pH 5.5
fast pH 5.5
slow pH 7.4
fast pH 7.4

**Figure 38**

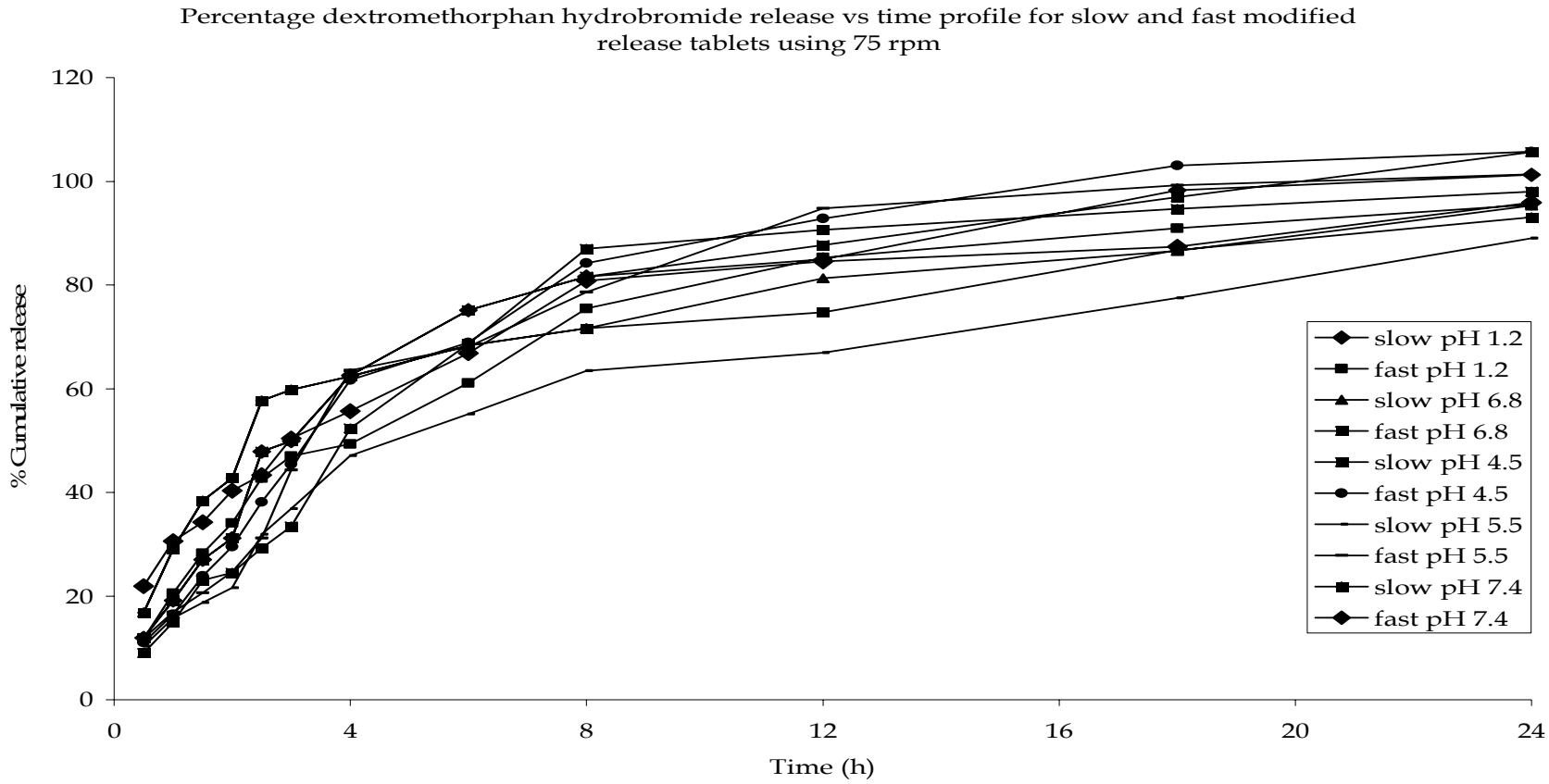

Percentage dextromethorphan hydrobromide release vs time profile for slow and fast modified release tablets using 75 rpm
% Cumulative release
Time (h)
0
20
40
60
80
100
120
0
4
8
12
16
20
24
slow pH 1.2
fast pH 1.2
slow pH 6.8
fast pH 6.8
slow pH 4.5
fast pH 4.5
slow pH 5.5
fast pH 5.5
slow pH 7.4
fast pH 7.4

**Table 62 IVIVC model linear regression of % absorbed versus % dissolved for Dextromethorphan hydrobromide tablets using pH 7.4 at 50 rpm**

| Time | Percentage dissolved (pH 7.4) | | Percentage absorbed | |
|---|---|---|---|---|
| | **Slow** | **Fast** | **Slow** | **Fast** |
| 0.0 | 0.0 | 0.0 | 0.00 | 0.00 |
| 0.5 | 9.31 | 13.98 | 22.90 | 20.74 |
| 1 | 15.21 | 33.21 | 32.91 | 29.44 |
| 1.5 | 21.35 | 45.70 | 44.63 | 39.36 |
| 2 | 27.20 | 53.42 | 58.17 | 50.48 |
| 2.5 | 38.77 | 56.22 | 67.97 | 62.99 |
| 3 | 39.20 | 58.32 | 76.36 | 71.29 |
| 4 | 44.08 | 70.94 | 90.13 | 84.00 |
| 6 | 59.64 | 87.26 | 104.06 | 97.03 |
| 8 | 68.59 | 93.58 | 103.10 | 97.38 |
| 12 | 81.47 | 98.56 | 94.73 | 98.38 |
| 18 | 88.44 | 102.39 | 98.82 | 98.12 |
| 24 | 94.29 | 102.87 | 100.76 | 99.92 |

**Table 63 IVIVC model linear regression of % absorbed versus % dissolved for Dextromethorphan hydrobromide tablets using pH 7.4 at 75 rpm**

| Time | Percentage dissolved (pH 7.4) | | Percentage absorbed | |
|---|---|---|---|---|
| | **Slow** | **Fast** | **Slow** | **Fast** |
| **0.0** | **0.0** | **0.0** | **0.00** | **0.00** |
| 0.5 | 16.83 | 11.89 | 22.90 | 20.74 |
| 1 | 29.06 | 19.18 | 32.91 | 29.44 |
| 1.5 | 38.42 | 26.98 | 44.63 | 39.36 |
| 2 | 42.72 | 31.16 | 58.17 | 50.48 |
| 2.5 | 57.67 | 47.85 | 67.97 | 62.99 |
| 3 | 59.83 | 49.90 | 76.36 | 71.29 |
| 4 | 62.40 | 72.54 | 90.13 | 84.00 |
| 6 | 68.34 | 85.15 | 104.06 | 97.03 |
| 8 | 71.63 | 91.61 | 103.10 | 97.38 |
| 12 | 74.74 | 94.98 | 94.73 | 98.38 |
| 18 | 86.73 | 98.28 | 98.82 | 98.12 |
| 24 | 93.02 | 101.30 | 100.76 | 99.92 |

**Figure 39**

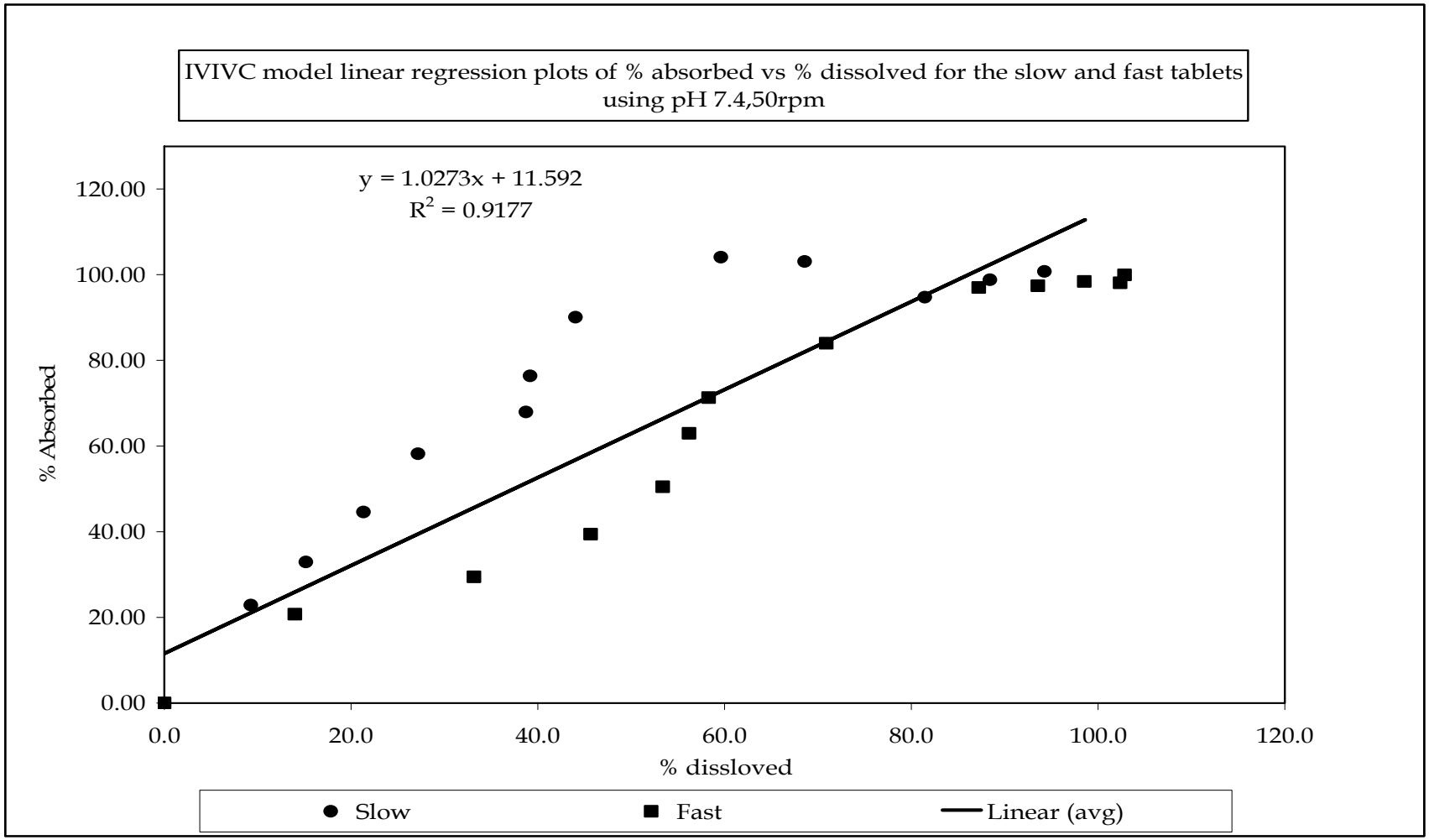

IVIVC model linear regression plots of % absorbed vs % dissolved for the slow and fast tablets using pH 7.4,50rpm
y = 1.0273x + 11.592
R2 = 0.9177
% Absorbed
0.00
20.00
40.00
60.00
80.00
100.00
120.00
0.0
20.0
40.0
60.0
80.0
100.0
120.0
% dissloved
Slow
Fast
Linear (avg)

**Figure 40**

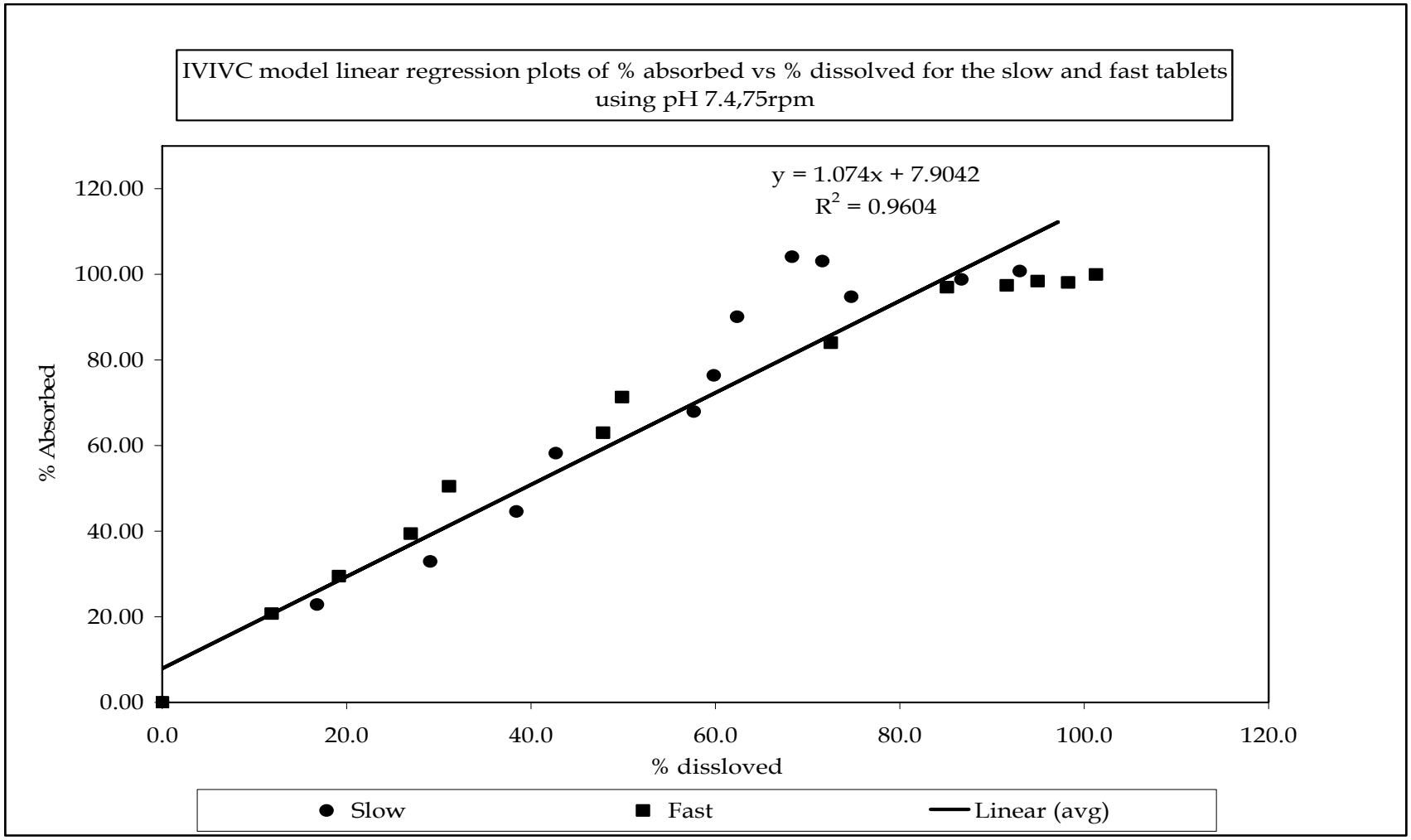

IVIVC model linear regression plots of % absorbed vs % dissolved for the slow and fast tablets using pH 7.4,75rpm
y = 1.074x + 7.9042
R2 = 0.9604
% Absorbed
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0.0
20.0
40.0
60.0
80.0
100.0
120.0
% dissloved
Slow
Fast
Linear (avg)

**Figure 41**

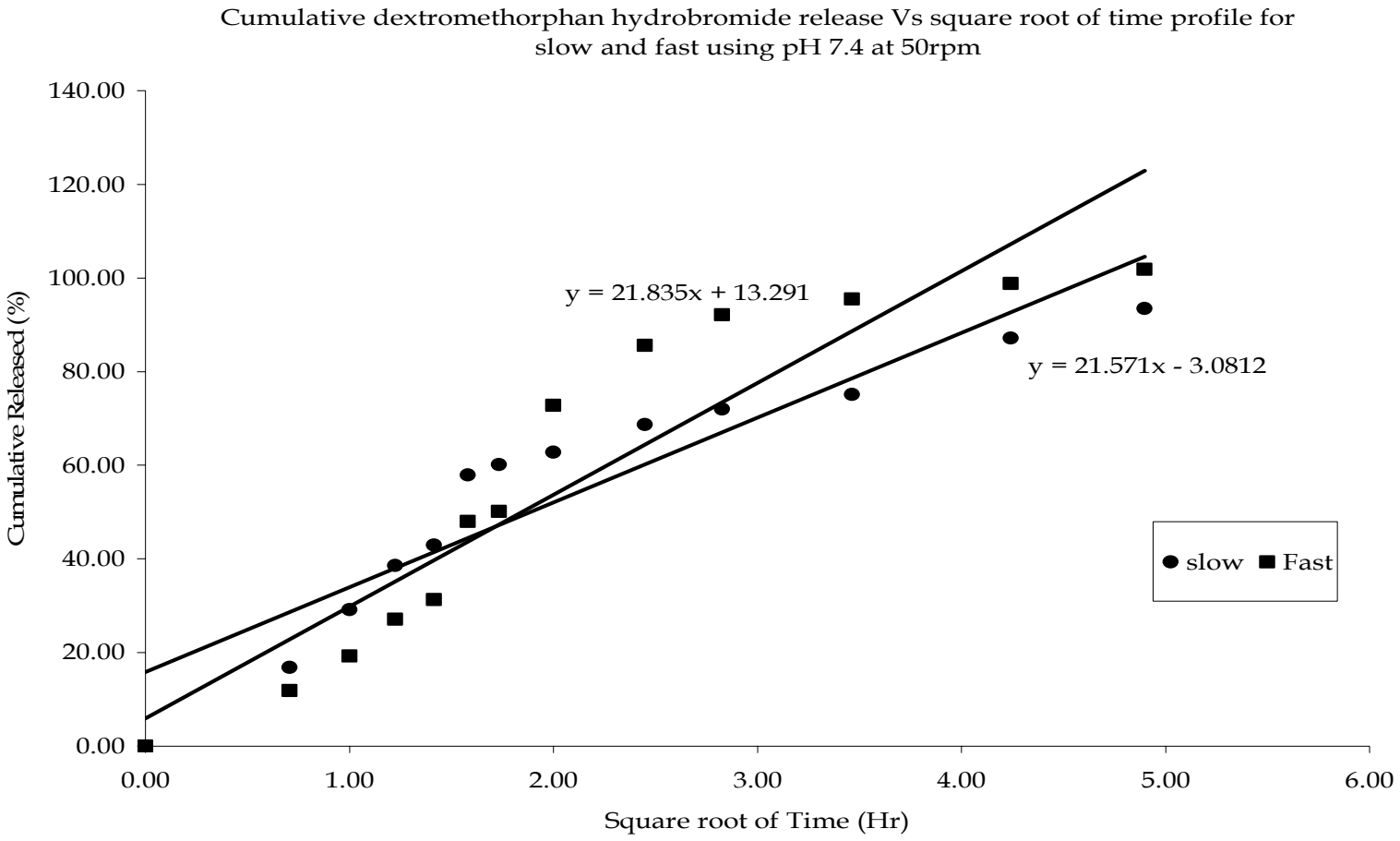

Cumulative dextromethorphan hydrobromide release Vs square root of time profile for slow and fast using pH 7.4 at 50rpm
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Cumulative Released (%)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Square root of Time (Hr)
y = 21.835x + 13.291
y = 21.571x - 3.0812
slow
Fast

**Figure 42**

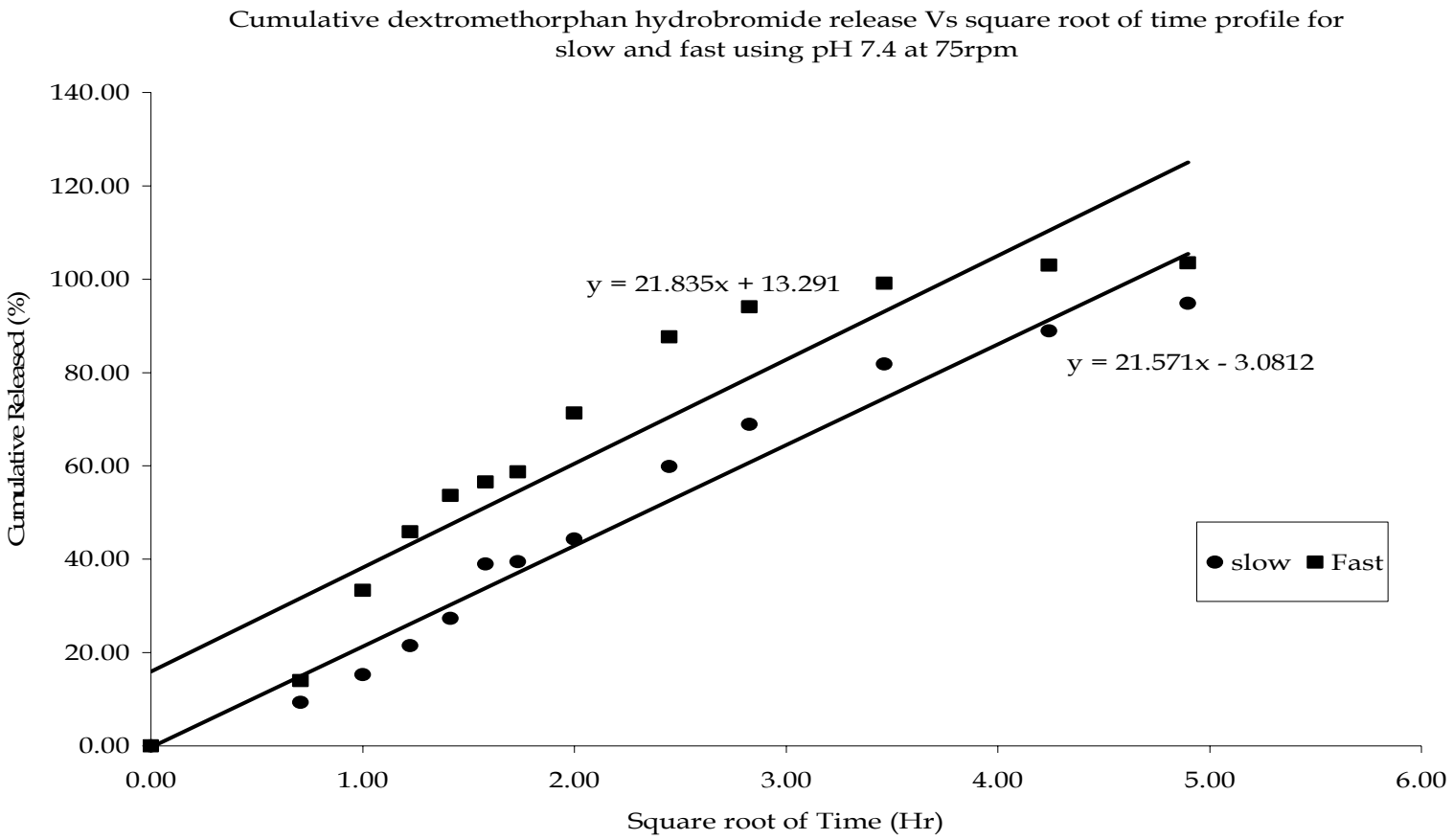

Cumulative dextromethorphan hydrobromide release Vs square root of time profile for slow and fast using pH 7.4 at 75rpm
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Cumulative Released (%)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Square root of Time (Hr)
y = 21.835x + 13.291
y = 21.571x - 3.0812
slow
Fast

**Figure 43**

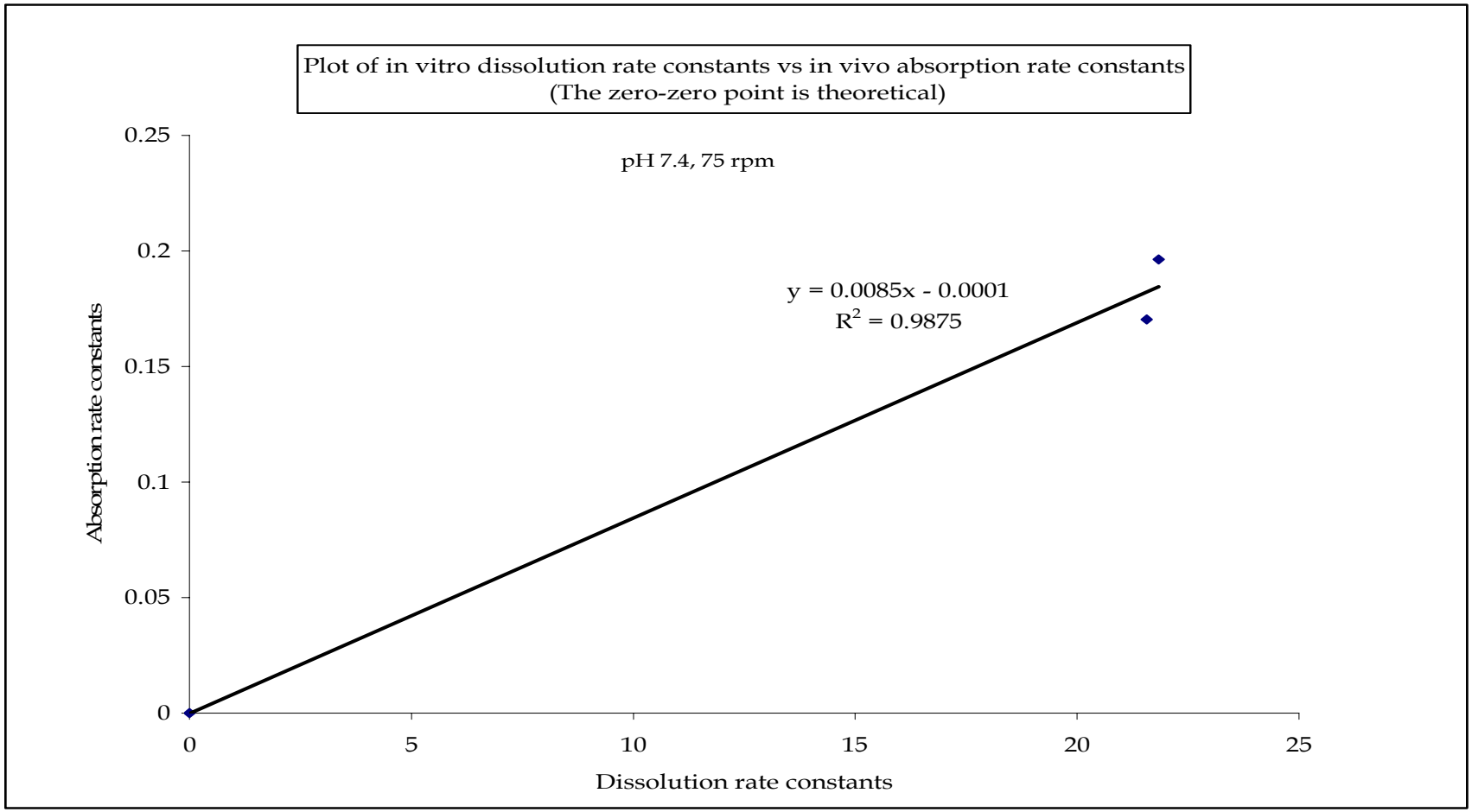

Plot of in vitro dissolution rate constants vs in vivo absorption rate constants
(The zero-zero point is theoretical)
pH 7.4, 75 rpm
y = 0.0085x - 0.0001
R2 = 0.9875
Absorption rate constants
Dissolution rate constants
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25

**Table 64 Observed and IVIVC model predicted $C_{max}$ and AUC values for Dextromethorphan hydrobromide**

| Time (Hours) | Slow formulation | | Fast formulation | |
|---|---|---|---|---|
| | Fraction observed | Fraction predicted | Fraction observed | Fraction predicted |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.50 | 590.29 | 238.27 | 613.667 | 134.75 |
| 1.00 | 827.11 | 580.92 | 847.431 | 343.92 |
| 1.50 | 1099.56 | 1008.84 | 1108.546 | 623.70 |
| 2.00 | 1409.05 | 1502.71 | 1394.795 | 967.12 |
| 2.50 | 1611.04 | 1964.67 | 1711.424 | 1521.07 |
| 3.00 | 1769.50 | 2202.29 | 1891.284 | 1915.87 |
| 4.00 | 1993.32 | 2206.11 | 2118.199 | 2354.36 |
| 6.00 | 2062.12 | 1831.54 | 2175.319 | 2361.35 |
| 00 | 1757.24 | 1591.02 | 1869.885 | 1860.99 |
| 12.00 | 783.48 | 661.85 | 1390.184 | 1319.40 |
| 18.00 | 296.67 | 197.09 | 732.045 | 437.16 |
| 24.00 | 189.73 | 148.98 | 197.520 | 151.17 |
| **AUC** | **22748.21** | **20983.45** | **29329.74** | **26742.92** |
| **$C_{max}$** | **2062.12** | **2206.11** | **2175.32** | **2361.35** |

**Table 65 Prediction errors (%) associated with $C_{max}$ and AUC for Dextromethorphan hydrobromide**

| Formulation | $C_{max}$ | AUC |
|---|---|---|
| Slow | -6.98 | 7.76 |
| Fast | -8.55 | 8.82 |
| Average | -7.765 | 8.29 |

**Figure 44**

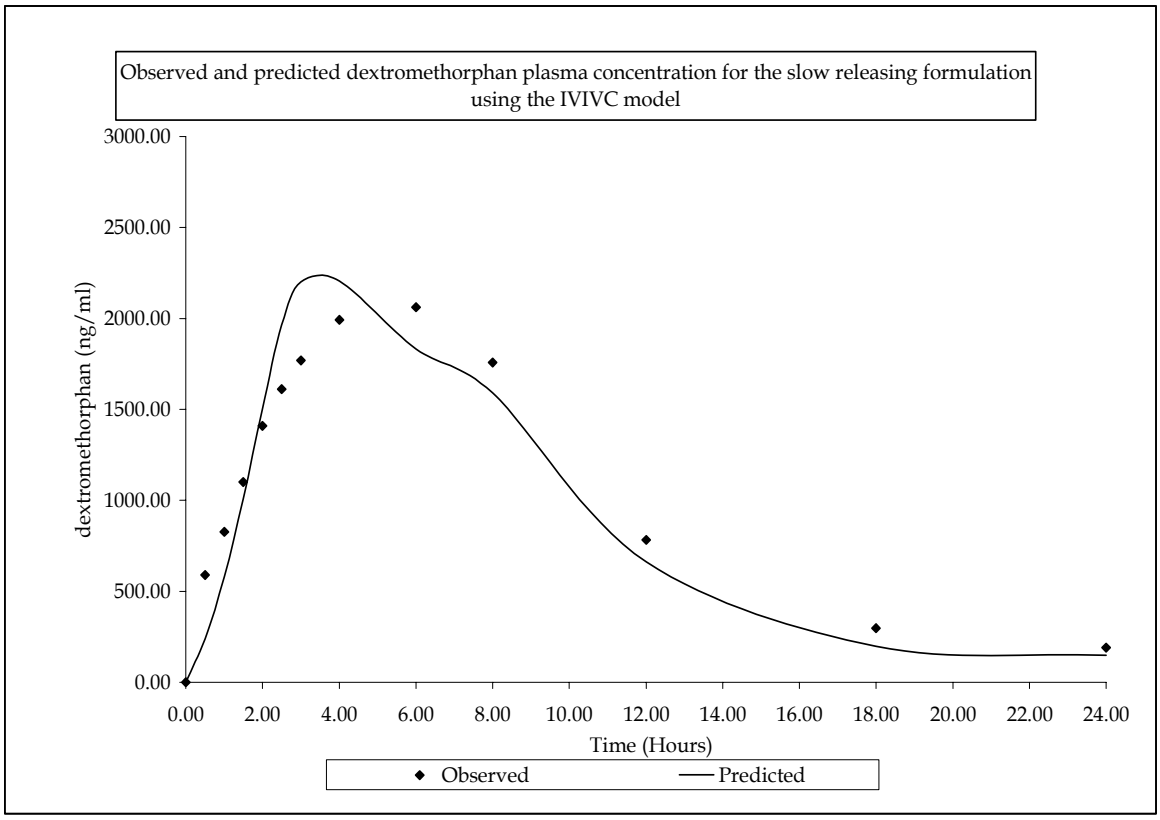
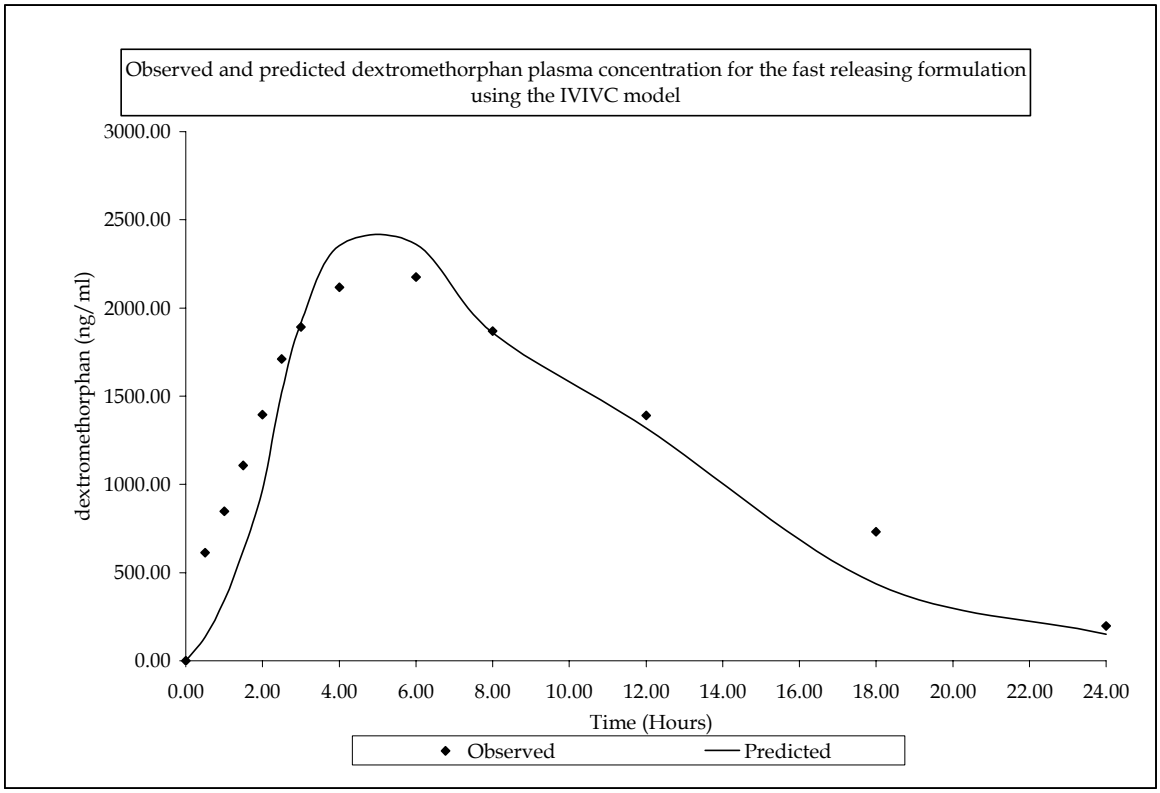

Observed and predicted dextromethorphan plasma concentration for the slow releasing formulation using the IVIVC model
dextromethorphan (ng/ml)
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Time (Hours)
Observed
Predicted

**Figure 45**


Observed and predicted dextromethorphan plasma concentration for the fast releasing formulation using the IVIVC model
dextromethorphan (ng/ml)
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Time (Hours)
Observed
Predicted

## 8. DISCUSSION

Manufacture of the selected MR formulations under different conditions (such as changes in batch size and manufacturing processes) to yield products with similar *in vitro* drug release profiles, in an IVIVC test method with acceptable internal predictability, shall be considered to exhibit "similar *in vivo* performance." Under current regulatory practice, this inference could have been reached simply on the knowledge of an acceptable internal predictability of the IVIVC. This report evaluated this practice by evaluating the external predictability of the IVIVC for selected manufacturing changes to the same formulation and also for a different formulation (different release mechanism). Results of this evaluation are in general agreement with current regulatory practice that recommends the use of an IVIVC with acceptable internal predictability to justify certain manufacturing changes (except for drugs with narrow therapeutic index, for which a demonstration of acceptable external predictability is recommended), and does not recommend the use of an IVIVC when a change in drug release mechanism is anticipated.

# 9. SUMMARY AND CONCLUSIONS

This thesis deals with the studies carried out by the writer for the past three years on the "Development and validation of *in vitro-in vivo* correlations for some modified release formulations".

Thesis begins with a brief account of the *in vitro - in vivo* correlations, biopharmaceutical classification systems, IVIVC models, *in vitro* dissolutions and estimation of drugs in biological medium. The methods used for the IVIVC model development, validation, the steps involved in bio analytical method development, *in vitro* dissolution methods and their importance have also been discussed. A review of literature on IVIVC model development available for the drugs in biological fluids is presented.

Thesis deals with the scope and objective of the present investigation. The merits of IVIVC in the development of dosage forms and how IVIVC model development necessitates development of *in vitro* dissolution methods, bio analytical method development and validation are discussed. The objectives of the present study, namely, to optimize the chromatographic conditions, to develop and validate the methods to estimate the selected drugs in the biological fluids by HPLC, development of *in vitro* dissolution methods and IVIVC model development and validation have been described.

Thesis also deals with the experimental procedures adopted. It describes in detail the procedures adopted for the bioequivalence study design & data handling, optimization and validation of the chromatographic conditions for the estimation of the drugs in plasma and selected modifies release (MR) formulations, IVIVC model development and validation.

The results obtained are presented, supported by tables and figures and discussed in detail. The discussions include,

- Bioavailability study design and data handling,
- Optimization and validation of the chromatographic conditions for the

estimation of the drugs in plasma and selected MR formulations are discussed such as,

- chromatograms obtained,
- accuracy,
- reproducibility (intraday and interday variations),
- specificity,
- linearity and range,
- LOD and LOQ,
- ruggedness and robustness,
- stability and
- system suitability studies.

➢ *In vivo – in vitro* data analysis

➢ *In vitro – in vivo* correlation

- model development
- validation

The following are some of the salient features of the present study;

i) A single dose, randomized, complete and three treatments cross over study was conducted in healthy human subjects and plasma concentrations were estimated by a sensitive, validated methods.

ii) The selected drug candidates ondansetron hydrochloride and dextromethorphan hydrobromide that are predominantly ionized at gastrointestinal pH ranges and are well absorbed after oral administration.

iii) The selected drugs can be categorized as high solubility/high permeability drugs under the proposed Class I and II Biopharmaceutical Classification System (BCS) and hence it should be possible to determine the *in vitro–in vivo* correlation for these drugs.

iv) The target to find out a predictive *in vitro* dissolution method was reached gradually. The first step was taken by observing the *in vitro* dissolution method predicted best similarities and differences in bioavailability. Apparatus I, pH 4.5 at 50 rpm was found to yield acceptable IVIVC for ondansetron hydrochloride and Apparatus I, pH 7.4 at 75 rpm was found to yield acceptable IVIVC for dextromethorphan hydrobromide.

v) From a comparison of the differences in the *in vivo* pharmacokinetic parameters and the differences in the *in vitro* dissolution curves, it may be concluded that the developed dissolution method will discriminate bio in equivalent batches.

vi) Level A correlation was observed for the selected formulations at the *in vitro* dissolution conditions developed. These dissolution methods predicted also the best absorption rate for the selected MR formulations.

vii) The validity of the correlation was also assessed by determining how well the IVIVC model could predict the rate and extent of absorption as characterized by $C_{max}$ and AUC. The percent prediction error of ≤ 10 % for $C_{max}$ and AUC was obtained, which establishes the predictability of the developed IVIVC model. It may, thus, be concluded that the developed dissolution methods can surrogate for human bioequivalence studies.

In conclusion, it may be pointed out that the developed *in vitro* dissolution methods can replace absorption studies during the pre-approval process to develop a desirable formulation and to ensure batch-to-batch bioequivalence. It will also be very useful in performing possible post-approval changes in the formulation scale-up or changes in the drug substance or excipients supplier.

**Recommendations**

This *In Vitro- In Vivo* correlations (IVIVC) have been applied for setting bio-relevant dissolution specifications, guiding new product development, supporting Scale-Up and Post Approval Changes (SUPAC), waiving bioequivalence study and more importantly, ensuring commercial product quality over the years. However,

further investigations in human are required to prove the clinical usability of the experimental extended-release formulation.

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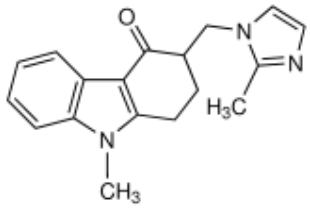
**Drug Profile of Ondansetron hydrochloride**

Ondansetron hydrochloride is the racemic form of ondansetron and a selective blocking agent of the serotonin 5-$HT_3$ receptor type. Chemically it is (±) 1, 2, 3, 9 – tetrahydro – 9 – methyl – 3 - [(2-methyl - 1H – imidazol – 1 -yl) methyl] - 4H -carbazol-4-one, monohydrochloride, dihydrate. It has the following structural formula:




The empirical formula is $C_{18}$ $H_{19}$ $N_3$ O•HCl•$2H_2O$, representing a molecular weight of 365.9.

Ondansetron Hcl dihydrate is a white to off-white powder is soluble in water and normal saline.

**Clinical Pharmacology**

**Pharmacodynamics:** Ondansetron is a selective 5-$HT_3$ receptor antagonist. While its mechanism of action has not been fully characterized, ondansetron is not a dopamine-receptor antagonist. Serotonin receptors of the 5-$HT_3$ type are present both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. It is not certain whether ondansetron's antiemetic action is mediated centrally, peripherally, or in both sites. However, cytotoxic chemotherapy appears to be associated with release of serotonin from the enterochromaffin cells of the small intestine. In humans, urinary 5-HIAA (5-hydroxy indole acetic acid) excretion increases after cisplatin administration in parallel with the onset of emesis. The released serotonin may stimulate the vagal afferents through the 5-$HT_3$ receptors and initiate the vomiting reflex.

In animals, the emetic response to cisplatin can be prevented by pre treatment with an inhibitor of serotonin synthesis, bilateral abdominal vagotomy and greater splanchnic nerve section, or pretreatment with a serotonin 5-HT3 receptor antagonist.

In normal volunteers, single intravenous doses of 0.15 mg/kg of ondansetron had no effect on esophageal motility, gastric motility, lower esophageal sphincter pressure or small intestinal transit time. Multi day administration of ondansetron has been shown to slow colonic transit in normal volunteers. Ondansetron has no effect on plasma prolactin concentrations.

Ondansetron does not alter the respiratory depressant effects produced by alfentanil or the degree of neuromuscular blockade produced by atracurium. Interactions with general or local anesthetics have not been studied.

- **Pharmacokinetics:**

Ondansetron is well absorbed from the gastrointestinal tract and undergoes some first-pass metabolism. Mean bioavailability in healthy subjects, following administration of a single 8-mg tablet, is approximately 56%.

Ondansetron systemic exposure does not increase proportionately to dose. AUC from a 16-mg tablet was 24% greater than predicted from an 8-mg tablet dose. This may reflect some reduction of first-pass metabolism at higher oral doses. Bioavailability is also slightly enhanced by the presence of food but unaffected by antacids.

Ondansetron is extensively metabolized in humans, with approximately 5% of a radio labeled dose recovered as the parent compound from the urine. The primary metabolic pathway is hydroxylation on the indole ring followed by subsequent glucuronide or sulfate conjugation. Although some non conjugated metabolites have pharmacological activity, these are not found in plasma at concentrations likely to significantly contribute to the biological activity of ondansetron.

*In vitro* metabolism studies have shown that ondansetron is a substrate for human hepatic cytochrome P- 450 enzymes, including $CYP_1A_2$, $CYP_2D_6$ and $CYP_3A_4$. In terms of overall ondansetron turnover, $CYP_3A_4$ played the

predominant role. Because of the multiplicity of metabolic enzymes capable of metabolizing ondansetron, it is likely that inhibition or loss of one enzyme (e.g., $CYP_2D_6$ genetic deficiency) will be compensated by others and may result in little change in overall rates of ondansetron elimination. Ondansetron elimination may be affected by cytochrome P - 450 inducers. In a pharmacokinetic study of 16 epileptic patients maintained chronically on $CYP_3A_4$ inducers, carbamazepine or phenytoin, reduction in AUC, $C_{max}$ and $t_{½}$ of ondansetron was observed. This resulted in a significant increase in clearance. However, on the basis of available data, no dosage adjustment for ondansetron is recommended.

In humans, carmustine, etoposide and cisplatin do not affect the pharmacokinetics of ondansetron.

Gender differences were shown in the disposition of ondansetron given as a single dose. The extent and rate of ondansetron's absorption is greater in women than men. Slower clearance in women, a smaller apparent volume of distribution (adjusted for weight) and higher absolute bioavailability resulted in higher plasma ondansetron levels. These higher plasma levels may in part be explained by differences in body weight between men and women. It is not known whether these gender-related differences were clinically important. A reduction in clearance and increase in elimination half-life are seen in patients over 75 years of age. In clinical trials with cancer patients, safety and efficacy was similar in patients over 65 years of age and those under 65 years of age; there was an insufficient number of patients over 75 years of age to permit conclusions in that age-group. No dosage adjustment is recommended in the elderly.

In patients with mild-to-moderate hepatic impairment, clearance is reduced 2-fold and mean half-life is increased to 11.6 hours compared to 5.7 hours in normals. In patients with severe hepatic impairment, clearance is reduced 2-fold to 3-fold and apparent volume of distribution is increased with a resultant increase in half-life to 20 hours. In patients with severe hepatic impairment, a total daily dose of 8 mg should not be exceeded.

Due to the very small contribution (5%) of renal clearance to the overall clearance, renal impairment was not expected to significantly influence the total clearance of ondansetron. However, ondansetron oral mean plasma clearance was reduced by about 50% in patients with severe renal impairment (creatinine clearance <30 ml/min). This reduction in clearance is variable and was not consistent with an increase in half-life. No reduction in dose or dosing frequency in these patients is warranted.

Plasma protein binding of ondansetron as measured *in vitro* was 70% to 76% over the concentration range of 10 to 500 ng/ml. Circulating drug also distributes into erythrocytes.

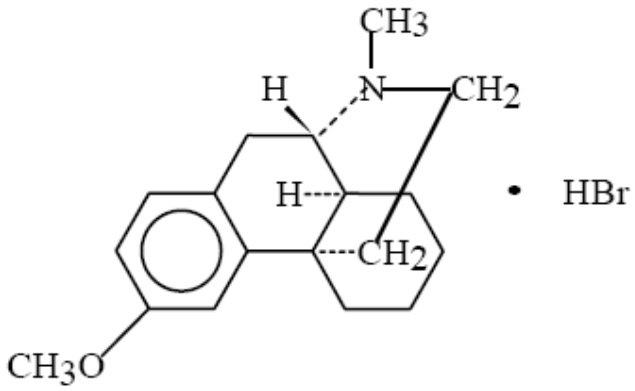
**INDICATIONS AND USAGE**

1. Prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy, including cisplatin ≥50 mg/m2.
2. Prevention of nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy.
3. Prevention of nausea and vomiting associated with radiotherapy in patients receiving either total body irradiation, single high-dose fraction to the abdomen or daily fractions to the abdomen.
4. Prevention of postoperative nausea and/or vomiting. As with other antiemetics, routine prophylaxis is not recommended for patients in whom there is little expectation that nausea and/or vomiting will occur postoperatively. In patients where nausea and/or vomiting must be avoided postoperatively.

**Drug Profile of Dextromethorphan**

Dextromethorphan (DM) was first reported in 1953 as an effective treatment of cough without the undesirable side effects of codeine like drowsiness, nausea and constipation. Since that time, dextromethorphan has become the active ingredient in many over the counter (OTC) products for treatment of cough due to upper respiratory infection like the common cold. Dextromethorphan is a safe and effective anti tussive agent.

**Physiochemical properties of dextromethorphan hydrobromide**




Molecular Formula: $C_{18}H_{25}NO,HBr,H_20$

Molecular Weight: 370.3

It is a white or almost white crystalline powder, with a faint odour. It is soluble in 60 (BP) or 65 (USP) parts water and 1 in 10 parts alcohol; freely soluble in chloroform with the separation of water; practically insoluble in ether. A 1% solution in water has a pH of 5.2 to 6.5. It should be stored in airtight containers.

***In vivo* studies of dextromethorphan**

Dextromethorphan has been extensively investigated in animals for study of toxicity and pharmacology. Effective antitussive activity has been repeatedly demonstrated in experimental cough in several species (guinea-pigs, rabbits, cats and dogs). Therapeutic doses have not been shown to cause respiratory depression, inhibition of ciliary activity, ataxia, lethargy, or sleep. Toxicity is rare at therapeutic doses with signs of adverse effects (mild sedation/ataxia)

appearing with doses of 20 mg/kg providing a large safety factor. Sub chronic evaluation of high doses over a 6-month period was without significant adverse effects.

**Pharmacology of dextromethorphan**

Dextromethorphan is the dextro-isomer of levorphanol, a non-narcotic codeine analog with little analgesic or addictive properties. It is thought to act on the cough center in the medulla oblongata by direct suppression of the cough reflex. Dextromethorphan is also thought to bind to two sites in the brain, high and low affinity sites which are distinct from opioid and other neurotransmitter binding sites. A steric hindrance mechanism may exist where the (O) methylated (+) form of racemorphan (dextromethorphan) prevents binding to the analgesic/addictive receptors in the medulla to abate the narcotic side effects. The pKa of dextromethorphan has been reported to be 9.12.

**Pharmacokinetic parameters of dextromethorphan**

| | |
|---|---|
| Elimination half life ($t_{1/2}$) (h) | 2.7 |
| Terminal disposition rate constant ($k_{el}$) ($h^{-1}$) | 0.2566 |
| Apparent volume of distribution ($V_d$) (1/kg) | 1.1 |
| Fraction of Unchanged Drug Excreted in urine ($f_{el}$) | 0.2 |
| Fraction of drug absorbed or absolute bioavailability (f) | 0.75 |
| Ionization Constant (pKa) | 9.12 |
| Therapeutic range or minimum Effective concentration (µg/ml) | 0.2 - 0.35 |
| Dose size (mg) | 30 |
| Dosing interval (h) | 6 - 8 |
| Time to reach peak ($t_{max}$) (h) | 2 |

After oral administration, dextromethorphan is rapidly absorbed from the gastrointestinal tract where onset of pharmacologic activity is between 15– 30 minutes and peak serum levels are achieved within 2.5 h. A controlled-release suspension containing 60 mg dextromethorphan given twice daily was bioequivalent to an immediate release solution containing 30 mg dextromethorphan given four times daily in slow and intermediate dextromethorphan metabolizers.

Dextromethorphan undergoes first-pass metabolism to O- and N demethylated metabolites including dextrorphan, the O-demethylated metabolite with antitussive activity. Another metabolite is 3-methoxymorphinan. Metabolism of dextromethorphan involves the oxidative enzyme cytochrome $P_{45}02D_6$ (or $CYP_2D_6$), for which activity is genetically determined and has polymorphic distribution in most populations studied. It has been estimated that approximately 10% of Caucasians in North America, Europe and Australia are poor dextromethorphan metabolizers in which DM persists in the plasma and is relatively slowly metabolized to DT. The dextromethorphan metabolic polymorphism is determined by the molar ratio of dextromethorphan to dextrorphan in the urine after a single dose administration of dextromethorphan such as urine molar dextromethorphan to dextrorophan ratio > 0.3 and ≤ 0.3 are indicative of slow and fast dextromethorphan metabolizers, respectively. Elimination half-life of dextromethorphan is 2 to 4 hours in the majority of individuals but may be as long as 28-74 h in slow metabolizers. No difference between fast and slow dextromethorphan metabolizers was reported for capsaicin-induced cough frequency. In contrast, slow dextromethorphan metabolizers had twice the citric acid administered (CAA) induced cough threshold observed in fast dextromethorphan metabolizers. To date, no published reports of increased incidence or severity of adverse events in slow metabolizers relative to fast metabolizers. The effects of liver disease on dextromethorphan oxidation was studied in 107 subjects and found that liver disease did impair dextromethorphan O demethylation, but to a much less extent than that observed in slow dextromethorphan metabolizers.

**Safety and dosage**

Dextromethorphan is dosed orally to adults at 10 to 20 mg every 4 h, or 30 mg every 6–8 h, to a maximum of 120 mg in 24 h (PDR, 2005). Children aged 6–12 years may be given 5–15 mg every 4–8 h to a maximum of 60 mg in 24 h, and children aged 2 to 6 years 2.5–5 mg every 4 h, or 7.5 mg every 6 to 8 h, to a maximum of 30 mg in 24 h. Dextromethorphan polistirex (Delsym®) (a dextromethorphan and sulphonated diethenylbenzene copolymer complex) is used in controlled-release preparations. Animal toxicity and clinical efficacy studies with dextromethorphan indicate that single doses of up to 120 mg/day produce few adverse effects which are usually minor and reversible. Ingestion of less than 10 mg/kg is unlikely to produce toxicity in a child. Long-acting preparations may have greater potential for toxicity in children. A study evaluating repeated dosing of dextromethorphan with 75 mg/day for 32 days was fairly well tolerated by subjects, with only 3 of 20 subjects reporting nausea, vomiting and dizziness.